

Europe's research postponed

This week's decision by the European Commission to withdraw its proposals for spending on new research creates a crisis (not for the first time), but one from which everybody might eventually profit.

THE doctrine that every cloud has a silver lining is often falsified by experience, but the European Commission's intemperate decision this week to suspend consideration of its plans for spending research funds in the current cycle is unlikely to be such an occasion (see page 177). At the very least, the incident, and the hiatus in research spending that will now follow, will draw attention to the circumstance that the European Communities (EC) have a research programme, may persuade people in European capitals (not to mention those in Brussels and Strasbourg) to pay attention to the way in which the programme is devised, and may even stimulate the long overdue discussion of how the programme should be managed and directed. That, potentially, is the silver lining.

The dark part of the cloud is the way in which the crisis has arisen, which hangs on the constitutional functions of the EC's several parts. There is the European Commission which, by definition in the Treaty of Rome, is the EC's civil service; it can make suggestions, even draft legislation, but it has no constitutional power of decision (but may acquire some through the indifference or neglect of its member states). Then there is the European Parliament (which oscillates physically between Strasbourg and Luxembourg), regarded by federalists as the eventual seat of European decision-making, but which now has powers only to approve of (or disapprove of) what is planned. Finally, there is the Council of Ministers, the appropriate ministerial representatives of governments who have traditionally called the shots, but whose wings have been clipped for the past five years by majority voting on a range of important issues.

Struggle

The long-term constitutional struggle, between the federalists and their opponents, is that between the Parliament and the Council; the Commission seems to have precipitated the row about research because it wishes to strengthen the arm of the Parliament, not that of the Council. Up to a point, that is a reasonable ambition: if Europe's civil servants were not natural federalists, would they be in Brussels? The trouble, for the Commission, is that it has chosen to make a row when the Council still has the whip-hand, at least on matters affecting its own power to decide. So the struggle between the federalists and their opponents, like that between those who believe there should be a constructive research pro-

gramme and those who do not care, is in danger of being buried by the ructions now in prospect.

Luckily, only a modicum of reason should suffice to bring sense to the EC's research programme, such as it is. Brussels now spends £700 million a year on research, and thus cajoles others into spending perhaps two-thirds as much. Most of this money goes on applied research, in information technology and telecommunications, where the ground-rules are that there must be at least two partners from different European countries and that the research must be "pre-competitive"; participants have usually to find half the cost. But the partnerships, often drummed up by hasty telephone conversations hours before a submission deadline, have frequently been mostly nominal. The judgement of what is pre-competitive has often been similarly bemusing.

Competition

In any case, by the end of 1992, competition rather than collaboration will be the order of the day. The Commission's judgements of what constitutes precompetitive publicly sponsored research should then be open to scrutiny from outside. Maybe the Commission's decision to withdraw the only five of its research programmes so far worked up in detail is an early recognition of this outcome, but that is far from probable. It may just have picked the wrong quarrel.

That is where the luck comes in. The Commission's decision to withdraw the few components of its research programme that had been essentially agreed must compel all those concerned — but especially the Parliament and the Council — to ask why European money is being spent on research at all. That is an interesting question. If the goal is really to run Intel, IBM, Fujitsu, Microsoft, Hitachi, Sony and the rest of them into the ground, the EC had better find a way of spending ten or twenty times as much. Otherwise, it should build for the more distant future. And that means making sure that Europe enables young people to be trained at being skilled. That means backing basic research. There is no choice.

Misfortune quickly follows. The Commission, inspired to that end by Commissioner Pandolfi, has only recently decided to abandon its only intellectually respectable programme for supporting research, called SCIENCE. Nobody breathes a word against the programme, but the Commission seems to take the line that if Pandolfi wants



something else instead — postdoctoral fellowships as it happens — he is entitled to them. But he is not, except by already moribund convention. By what right, but that of unbridled ego-gratification, can a national government's nominee discard one of its few conspicuous successes? The simple answer is that he needs none. The complicated answer is that the European Parliament, if it wants to plot a secure course for the years ahead, should ask itself how much of its present trouble stems from the foibles of the commissioner ostensibly in charge.

There are fortunately solutions. To let things run until the end of 1992 (when the Commission will be re-appointed by Europe's governments) will not do. The immediate need is that the European Parliament should be made to understand the complexity of the task ahead of Europe's research enterprise, which is not simply about making cheaper microchips or about keeping the concentrations of nitrate ions in drinking water ruinously below those barely allowed in the United States. The objective should not be innovation, but skill (of which even a little will go a long way). That should also be the Commission's objective, and the Commission (being intelligent but unthinking) may be a better target. Whatever the best tactics, the time has come when Europe's scientific community should say what Europe should be doing in research. Quickly and clearly.

The chief responsibility lies with Europe's universities. Although many of them, in recent decades, have been robbed of the autonomy they would usually wish to boast of, they are the only institutions that regularly engage in a dialogue with the young, and those best equipped to tell where the future lies. The universities are especially downcast in eastern Europe, outside the EC and likely to be impoverished for many years to come. But is it not the fate of university institutions to pay for the privileges they enjoy by speaking the unspeakable? Europe in the larger sense needs more than a network of productive enterprises, but an intellectual community. How will that be provided, and when? □

Balkanization again?

It will be a shame if Yugoslavia breaks up, but maybe the outcome will be free movement of academics.

THE political conundrum of the 1970s — "What will happen when Tito dies?" — seems to have been answered after an inordinate delay: balkanization, perhaps even chaos. Tito was the strongman communist who played a crucial part in ending the occupation of Yugoslavia during the Second World War and who then held the motley Balkans together by a blend of dictatorship and guile. His decision, early on, to follow a line independent of Moscow was a recognition both that the Soviet writ would not run easily in the Balkans and that there would be advantages, during the Cold War, in playing off the West against the East. But the post-Tito stratagem for keeping Yugo-

slavia together by rotating the presidency between the undemocratically elected heads of the six separate republics has now fallen apart, the victim of popular discontent with continuing backwardness, of ethnic rivalry and suspicion and of the corrosive influence of still more backward Albania, exercised through once-autonomous Kosovo. Yugoslavia will not readily be put together again.

So must Europe now look forward to the arrival of five extra members (six republics instead of one federation) of the international community? The best hope — for Yugoslavia — is some other solution. Yugoslavia has been unified now for 70 years, since the end of the First World War; unscrambling the federation would be far from simple. Yet the fissiparous tendencies of the past few years have already caused needless damage, notably to the federation's stripling but ingenious research enterprise. By the late 1970s, after responsibility for research had been concentrated at the centre on the old stalinist model, it was devolved to the republics in a manner almost calculated to waste even meagre funds. And it is too soon to know whether the restoration of some federal funds will be the palliative it was meant to be.

For a country in which respect for academic enterprise flourishes (the University of Belgrade has close on 60,000 students, Zagreb in Croatia two-thirds as many, while the republic of Slovenia, abutting Italy, should later this year have trained 2,000 people to PhD level), it will be a cruel disappointment if all the efforts of the past three-quarters of a century are wasted. For much of that time, Yugoslavia has been hoping that science and technology would eventually enable a largely rural population to leapfrog into the twentieth century. (Who remembers, now, Yugoslavia's 1960s' plans to build nuclear power stations?) On the face of things, 22 million people divided into six republics will find that goal even more elusive.

So it matters that the predicament of Yugoslavia is not unique. Depending on the outcome of last week's referendum in the Soviet Union and its aftermath, there could soon be a further seven near-sovereign European states (three Baltic republics, Georgia, Azerbaijan, Armenia and even, against the odds, Moldavia). Hopes that Slovakia will always be a part of Czechoslovakia are similarly fading. What could such a mixed bag of small countries hope separately to accomplish in science, and in scholarship more generally? The German experience with reunification shows that even Western pockets are not bottomless; newly independent European states will not find capital thrust upon them by their neighbours to the West. And their small size must impede self-improvement. Yet there could be ways round these formidable difficulties. It is only a century since the universities of Central Europe played host to peripatetic academics from the West. (Ernst Mach taught at Prague for much of his life, for example.) Even if governments cannot secure immediate economic help from the West, arranging for the free movement of academics from elsewhere would win them an even more valuable prize — a foundation for the future. □

'Civil war' scuttles EC research programme

- New Framework programme delayed
- Commission defies member states

London

THE European Communities' (EC) research programme has fallen victim to a power struggle over who controls EC research funding. The EC's civil service, the European Commission, announced last week that it has decided to withdraw five planned research programmes — a move that will block any spending on the EC's third four-year Framework research budget until at least late 1992. Spending on the third Framework was to have started this year.

The decision to withdraw the research programmes is the latest round in a fight between ministers from the EC member states and the Commission. But it is the most serious, as the Commission's latest move has the backing of the European Parliament. The row will delay programmes in marine science and technology, the environment, life sciences and technologies for developing countries, and two programmes in telecommunications — which are the only five among the 15 proposals that make up the third Framework to have made any significant progress down the tortuous path towards final approval.

The decision to withdraw the programmes, which was announced by the Commission's president Jacques Delors, had not yet been presented in writing as *Nature* went to press, but with feelings on both sides running high, a compromise to prevent the programmes' withdrawal seemed unlikely.

The Commission's complaint is that the member states' research ministers have altered the five programmes from the Commission's original proposals, and have refused to include amendments suggested by the Parliament. But the move is seen by many as part of a wider plan by the Commission and the Parliament to increase their own power within the EC. The strongest influence over EC decisions now lies with the ministers of member states, but the Commission and the Parliament both see an opportunity to increase their powers in the coming months.

The Commission's defiance comes as revisions to the EC's constitution are being considered, with member states debating plans for greater EC political and economic unity. Antonio La Pergola, chairman of the Parliament's Energy, Research and Technology Committee, last week welcomed the decision, claiming it as "a step towards restoring the balance" between the member states, the Commission and the Parliament in EC decision-making.

The Commission is now expected to

rewrite the five proposals, before submitting them again to the member states' research ministers. This will delay the spending of more than 1,000 million ECU (about £700 million) in research grants. For some European scientists, particularly those involved with research aimed to benefit developing countries (where the second Framework budget is exhausted), the delay means that no EC money will be available for more than a year.

The disagreements over the five programmes cited by the Commission centre around their management, rather than their scientific content. For example, the research ministers removed a provision that would have allowed the Commission to distribute 10 per cent of each programme's budget to scientists whose research grant applications do not fit precisely within the terms of the formal request for applications. Another contentious issue is the research ministers' decision that the environment research programme should be overseen by a powerful management committee able to refer decisions taken by the Commission back to the research ministers for approval. The Parliament and the Commission say that the committee should have an advisory role only.

Representatives of the member states in Brussels are shocked by the Commission's unprecedented move, and question its legality. The Commission is allowed to withdraw research programmes, but only if the proposals have been changed substantially ('denatured', in Euro-jargon) by the member states' ministers. The ministers' legal advisers believe this is not the case for the five programmes in question, and the ministers may take the Commission to the European Court of Justice. But although that may prove a legal point, it is unlikely to accelerate spending of the third Framework budget.

Glyn Ford, a British Labour Member of the European Parliament who sits on La Pergola's Energy, Research and Technology Committee, says he has "some sympathy" with individual scientists who will be denied research grants over the coming year, but maintains that the Commission's move "is in the longer-term interest of EC research and development". Ford believes that the European Parliament, as an elected body, should have a stronger role in formulating the EC's research programme, and accuses the member states' ministers of "riding roughshod" over the wishes of the Parliament and the Commission.

Peter Aldhous

UK NUCLEAR PHYSICS

Daresbury hopes dashed

London

THE UK Science and Engineering Research Council (SERC) has announced that the tandem accelerator at its Daresbury laboratory will close at the end of 1992, ending nuclear physicists' hopes of obtaining extra funds to continue running the machine. The closure of the Daresbury Nuclear Structure Facility (NSF) will leave British nuclear structure researchers dependent on access to accelerators on the European mainland.

In the package of cuts unveiled by SERC last month, the NSF's survival beyond 1992 was made contingent on a successful bid for extra money for Daresbury from the 1992–93 science budget (see *Nature* 349, 551; 14 February 1991). But that rescue attempt has fallen at the first hurdle. SERC's announcement came only days after a weekend meeting of the Advisory Board for the Research Councils (ABRC), convened to discuss the research councils' bids for new money for 1992–93. SERC staff last week declined to comment on that confidential meeting, but it seems that the ABRC vetoed the bid for money to save the NSF.

Sir Mark Richmond, chairman of SERC, last week said the decision marks "a sad day for the SERC". SERC will try to minimize redundancies, but natural wastage and transfers to other laboratories seem unlikely to save all of the 150 NSF staff.

Until the number of forced redundancies is known, the cost to SERC of closing the NSF after 1992 cannot be calculated, but it is likely to amount to several million pounds if the building is to be demolished.

The announcement has demoralized British nuclear structure researchers, who had been led to believe that SERC would carry out a full scientific review of British nuclear structure physics before making a decision on the NSF. SERC staff say that a review of British nuclear structure physics will still take place, but this will now concentrate on research grant spending, currently running at about £3 million a year. Without their centrepiece facility, nuclear structure physicists fear that grant spending will soon be eroded.

Peter Twin, from the University of Liverpool, and chairman of SERC's Nuclear Structure Committee, describes the promise of a review as "hollow".

The priority now is to obtain time for British physicists on accelerators elsewhere in Europe. Twin warns that the initiative must be taken by SERC, not just the scientific community, if European science funding agencies are to be convinced to open their facilities to British researchers. The perception now is that SERC has "no policy" for nuclear structure research, Twin says.

Peter Aldhous

Healy ready to take on NIH

Washington

AFTER more than a year and a half of anxious anticipation, the US National Institutes of Health (NIH) are finally to get a leader. On 14 March, cardiologist Bernadine P. Healy, President George Bush's nominee for the NIH directorship, sailed through confirmation hearings before a Senate committee chaired by Edward M. Kennedy. Final approval of the full Senate is considered certain, which means that Healy may actually take office within the next few weeks.

Described by those who have worked with her as "smart", "very traditional", "strong-willed" and a "tough bird", Healy seemed eager to take on the challenge of guiding the NIH through what biomedical scientists argue are unusually hard times. She revealed something about what she thinks most important through what she chose to talk about in her testimony and responses during two hours of harmonious give and take. After months of prudent silence, the confirmation hearing marked Healy's first public comments on her views about the scientific enterprise.

Calling the NIH "a national treasure", Healy nonetheless made a point of telling the Senate committee that she does not think researchers have an entitlement to federal funding just because large numbers of them want support. Rather, she said, the United States has an obligation to fund NIH researchers at the home campus in Bethesda, Maryland, and at more than 1,700 institutions around the country and around the world, "not because they are entitled to it or because they always behave so well, but because it is the only way to fulfill our goals for a healthier world".

Healy, who is 47, is a graduate of Vassar College and Harvard Medical School, and trained at Johns Hopkins University, where she rose to the rank of professor of medicine. She spent nearly two years in the White House Office of Science and Technology Policy during Ronald Reagan's presidency, and comes to NIH from her current post as head of the Research Institute of the Cleveland Clinic Foundation.

The delay in finding a successor to former NIH director James B. Wyngaarden, who left the institutes in 1989, is connected to the role abortion plays at all levels of US politics. The Bush administration was under considerable pressure to appoint someone who is vocally opposed not only to abortion but also to the use of human fetal tissue in transplantation research. At least a couple of candidates rejected the job, or were rejected, over that issue.

Indeed, Healy's own nomination has come under attack because as a member of a presidentially appointed NIH study panel, she voted with the majority to allow fetal tissue research under certain carefully prescribed circumstances.

In the end, the matter was settled with a political compromise that one senator described this way: Healy will "make medical decisions free of political concerns", and will leave "policy to politicians". Although her written testimony before the Senate contained no mention of fetal research, she volunteered as an aside that she will support the administration's long-standing ban on these studies.

Saying that sometimes science needs to take "time out" when its goals collide with the moral and ethical concerns of the society, Healy cited a number of areas in which science has had to be accommodating — recombinant DNA research, animal research and the human genome project among them. The experienced politician in Healy said that "science indeed does not live by and unto itself alone".

Thus, in the vein of responding to public



Bernadine Healy wants to protect the Mozarts in modern-day science.

concern, Healy found herself in the convenient position of being able to endorse the politically popular idea that women's health deserves more attention from NIH. In response to a query from Maryland Senator Barbara Mikulski, Healy, long concerned about heart disease in women, said women's health generally is "one of my most favorite subjects". As the first NIH director to have a \$20 million discretionary fund, Healy said that she has already decided to allocate \$2–\$3 million of that money to the institutes' new office of women's health.

Tackling the crisis mentality that has arisen because NIH cannot fund more than

24 per cent of the deserving grants they review — a percentage Healy called "way too low" — she made plain her willingness to tackle what NIH call "cost management", but which is known to researchers as a system for cutting 10 per cent or more from their grants. NIH officials have been working on a plan to bring grant costs under control without approaching the touchy issue of indirect costs or overhead that is paid to universities to support such items as administration and libraries. It has become a matter of particular concern recently and the subject of congressional hearings (see page 182).

NIH cost planners, working without the leadership of a full-time director, told Healy they would not touch that politically sensitive issue. "Well, Senator", she declared, "I'm prepared to touch it." Nearly \$2,000 million of NIH's \$8,000 million annual budget goes to indirect costs which currently are now outside NIH's control. Indirect cost rates are now negotiated between the university and a federal auditor unconnected to

NIH, who operates according to rules written by the White House Office of Management and Budget (OMB). Healy wants to see NIH included in that negotiating process or in any revision of the OMB rules.

Technology transfer is another topic high on the new director's agenda. A strong advocate of collaboration among basic scientists, physicians, and industry, she favours the US push, under a 1986 law, actively to foster such research, despite instances of real or perceived conflict of interest that may arise as complex collaborative agreements are negotiated. "Those difficulties are not a reason to walk away from a principle of great social value", she said. While she sees the need for some flexibility in basic research agreements, she draws a stricter line when it comes to clinical medicine.

Having first-hand experience with conflict of interest over stock she once owned in Genentech, she has taken the lead in declaring that physicians should

have no financial ties to companies whose drugs or devices they are testing in patients.

Healy also chose to speak to the Senate rather poetically about scientific creativity. "There is a lesson for science in the play *Amadeus*", she offered. Mozart the creative genius, "magical and brilliant", was also "difficult, childish, nasty, and unconventional". On the other hand, his rival Salieri was "talented in a workmanlike way and popular at court". Said Healy, "If medicine is to succeed, the Mozarts must be allowed to flourish". She did not say whether she had any particular modern-day Mozart in mind, and no one asked.

Barbara J. Culliton

EUROPEAN RESEARCH

Gordon conference clones

Munich

IN an attempt to counteract a sense of being second best to the United States in setting the trends in scientific research, a group of European researchers from a wide variety of disciplines is borrowing an idea that has proved quite successful in boosting US science. The group, led by such eminent researchers as the German ethologist Hubert Markl, head of the granting agency DFG (Deutsche Forschungsgemeinschaft), and physicist Sir William Mitchell of Clarendon Laboratory in Oxford, is trying to develop an array of European Research Conferences modelled on the renowned Gordon Conferences in the United States.

If the proponents get the 10 million ECU (about \$7.7 million) over five years that they are requesting from the European Communities (EC), they will organize as many as

HEALTH AT WORK

Exoneration for VDTs

Washington

PREGNANT women who use video display terminals (VDTs) in their work are no more likely to suffer miscarriages than women who do not, according to a report published last week (*New England Journal of Medicine* 324, 728; 14 March 1991).

The study, which was carried out by researchers at the National Institute for Occupational Safety and Health (NIOSH) in Cincinnati, Ohio, should allay fears raised by clusters of spontaneous abortions among women who have worked with VDTs. Several miscarriages, for example, were reported in 1988 among women who worked at *USA Today* in Arlington, Virginia.

The NIOSH scientists studied two groups of women telephone operators in eight states in the southeastern United States. One group consisted of directory-assistance operators who used VDTs, and the second group was composed of general telephone operators who did not. There was no increased risk of miscarriage among the VDT users; indeed, they had a slightly lower rate of miscarriage, although the difference was not statistically significant.

The results appear to rule out very-low-frequency (15 kilohertz) electromagnetic fields as a risk factor in causing spontaneous abortions, as operators who used VDTs had measurably higher exposure to these fields than did the control group. The researchers noted, however, that the data say nothing about risks associated with exposure to extremely low frequency (in this case, 45–60 hertz) fields. The measured exposure levels to these fields were approximately the same for both groups of operators.

Robert Pool

120 conferences a year by 1995 in five major fields: chemistry, physics, biosciences, geosciences and humanities.

The conferences, which will be overseen by the Strasbourg-based European Science Foundation (ESF) in conjunction with several European scientific societies, will in some cases merely continue a tradition of European cooperation established by scientific societies. The European Chemistry Society, for example, has been running pan-European meetings for many years. But the planned conferences would be broader in scientific scope and in their degree of international participation than any existing European programme.

Perhaps the most important innovation will be the attempt to include large numbers of young researchers from all regions of Europe in the conferences. By making EC money available for the conferences, it will be possible for groups in less-developed countries to send their junior members to international meetings.

A few meetings of this type have already been held by the ESF and have met with an overwhelming response. One meeting, on supramolecular chemistry, set to be held later this year under the chairmanship of French Nobel laureate Jean-Marie Lehn, has received 250 requests for information even before the first public announcement.

Like the Gordon Conferences, which have been held annually for 60 years, mostly in New England, the European conferences would bring together a varied group of researchers for five-day meetings on narrowly defined subjects. Most subjects would be addressed by a series of meetings to be held every other year. The organizers would allow ample time for recreation and socializing in order to encourage the free exchange of ideas.

And as at the Gordon Conferences, researchers attending the new meetings would be asked not to discuss the content of the meeting with outsiders. This measure would encourage the participants to discuss even 'half-baked ideas' as well as recent, unpublished results from their laboratories, says Josip Hendekovic, executive secretary of ESF. It is precisely this kind of unpressured exchange that has made the Gordon Conferences so successful.

Although European researchers have sometimes been invited to speak in the United States, they often feel that they are excluded from the process of choosing what will become the 'fashionable' fields of science.

Hendekovic and the other backers hope that the conferences will increase a sense of European identity, especially among young researchers. He sees a time coming by the year 2000 when the United States will draw even more strongly on European and

AIDS RESEARCH

Inquiry launched into ethical procedures

Paris & Washington

THE French health minister, Bruno Durieux, has ordered an inquiry into research carried out by Daniel Zagury, an AIDS researcher at the Pierre and Marie Curie University in Paris. Zagury's research on AIDS vaccines, in collaboration with scientists at the US National Cancer Institute, including Robert Gallo, is the subject of a recent article by John Crewdson, a *Chicago Tribune* reporter, suggesting that ethical procedures may not have been respected. Zagury says that he followed appropriate ethical procedures for research in France and that he was not subject to US guidelines because he was not actually using materials from US laboratories.

The inquiry will initially focus on Zagury's work at the Saint-Antoine hospital in Paris, where two trials of a candidate AIDS vaccine (based on deactivated gp 120 protein) have been carried out on volunteers: one on seronegative medical interns, the second on patients suffering from AIDS. There is also controversy surrounding clinical trials carried out in Zaire by Zagury, but it is unlikely that these will be the object of the same French health ministry inquiry.

The US National Institutes of Health halted all collaboration with Zagury in early February and is conducting its own investigation into the case.

Peter Coles & Christopher Anderson

Japanese talent in science and engineering to fill a growing 'talent gap'. In order to prevent the top European researchers of tomorrow from migrating to the United States, Hendekovic says, Europe needs to start now to convince young people that they would not be "missing the boat" if they stayed in Europe.

If approved by the EC, the conferences will bring in primarily — but not exclusively — European participants, including some from countries that are not EC members. Switzerland, Sweden and other European Free Trade Association (EFTA) members, several of which have bilateral scientific cooperation agreements with EC, would participate, as well as ESF members such as Hungary, Yugoslavia and Turkey.

According to Hendekovic, optimists say that the EC will reach an agreement this summer on the conferences, which are included in line 6 of the budget for the third Framework Programme in the section on "human capital and mobility".

If the EC agrees this summer, the conferences could start in spring 1992. But delaying the decision, Hendekovic says, would make it "very, very difficult" to get the conferences started on the schedule ESF would like.

Steven Dickman

Water shortage pits man against nature

San Francisco

HEAVY rains and snows fell in California last week, bringing a welcome respite to this state that has now suffered five years of drought. As welcome as it was, however, the precipitation will not go very far to restore the withered landscapes, dry reservoirs and fallow farmland that have become all too common in California.

In the face of nature's adversity, engineers and planners are now turning to scientific and technological solutions to the water shortage. Instead of praying for rain, they are considering such options as seeding clouds over the Sierra Nevada mountains, desalting Pacific Ocean seawater and piping fresh water thousands of miles from Alaska.

Although it has rained little in California in recent years, the drought is not due to a lack of storms, says Tom Henderson, president of Atmospherics, Inc., a cloud-seeding company in Fresno.

The drought years have actually produced 80 per cent of the number of storms of normal years, he says. Instead, the problem is that the storms in wet years were generally taller, thicker, longer-lasting and much higher in liquid water content than those in the drought years. So, while some regions have seeded clouds for years to increase rainfall, this winter several more communities have turned to various cloud-seeding techniques to enhance the weaker storm systems.

Monterey County is paying about \$130,000 to send airplanes into every cloud that has the potential to produce rain for the area this season. The planes release tiny crystals of silver iodide which, at appropriate temperatures and sites within the cloud, act as nuclei for ice crystals, which later become rain or sleet or snow. Monterey County dis-

trict meteorologist John Stremel finds the initial results of the cloud-seeding programme "very encouraging", although actual increases will not be calculated until the end of the season.

Over the high hills of Santa Barbara, cloud seeding has led to a 25 per cent increase in rainfall, with rain falling earlier during storms and more intensely. Most efforts do less well, Henderson says — the process typically yields a 5–15 per cent increase in annual precipitation.

Silver iodide, used in 95 per cent of all cloud-seeding efforts worldwide, is the material of choice for rain-making, but researchers are testing a variety of other materials. Dry ice, for example, can be used when cloud-top temperatures are between -4°C and -5°C , warmer than the conditions for silver iodide. And Atmospherics is experimenting with different organic compounds that modify the seeding agents for use in different cloud conditions.

Henderson has also seeded a few clouds with the bacterium *Pseudomonas syringae*, which is usually found on plant leaves, where it nucleates ice crystals. Atmospherics scientists are still optimizing its use in laboratory tests, but they have found it can form crystals at the relatively high temperature of -0.5°C .

In the Plumas National Forest, the state Department of Water Resources and the US Forest Service are developing an experimental ground-based cloud-seeding programme that uses propane to trigger precipitation. Propane has been used by the US Air Force to clear fog at airports, but this project is expected to mark its first use in cloud-seeding. Courtland Bennett of the US Forest Service says the plan calls for ten seeding stations on mountain ridges over Feather River to

release liquid propane into the atmosphere at cloud level in the hope of generating additional runoff into reservoirs of the State Water Project.

Some California cities have begun to look toward a source of almost unlimited water: the sea. Although desalination plants have operated for years in Middle Eastern countries, and even in Florida in the United States, they have traditionally been considered too expensive for use in California because they require huge amounts of en-



ergy.

But the city of Santa Barbara has decided to tolerate the high cost in return for a guaranteed water supply. In that central coast city, where the two main water reservoirs are just 12 and 14 per cent full, officials say construction of a desalination plant will begin by midsummer. The city currently delivers water to its customers for \$200 per acre-foot, but it will pay contractors \$1,900 per acre-foot for the desalted water when it becomes available in a year. (An acre-foot of water is 326,000 gallons, or enough to supply a suburban family of five for a year.)

Desalination plans are also under scrutiny

Drought threatens Californian bird populations

CALIFORNIA'S drought is putting at risk several populations of the state's endangered and threatened species, including a pioneering pair of bald eagles and several types of fish.

At Lake Cachuma in central California, US Fish and Wildlife Service biologists are worried about a breeding pair of endangered bald eagles. Although this part of the state was once a common breeding ground for the eagles, the birds retreated north in the 1950s, and it was only two years ago that this pair became the first to recolonize the Lake Cachuma territory. But the lake, which supplies water to Santa Barbara, is just 14 per cent full, and biologists fear that if it loses its fish populations, it will also lose the pair of resident eagles as well as 15 other eagles that winter there before returning to their northern nesting grounds.

With only 2,000 breeding pairs of eagles in the continental United States, it is important to encourage the raptors to

colonize many areas so that a local disaster does not destroy the species, says Robert Mesta of the Fish and Wildlife Service. The Lake Cachuma pair "represent the seed of a Southern California population", he says, and their progeny will probably return to the lake.

Further north, in the Sacramento River delta, winter-run chinook salmon are in danger. For a number of reasons, the salmon population, which runs up the Sacramento River to spawn, has been diminishing for several years and last year reached a record low of 441. Now biologists are worried that after upstream water is diverted by federal water projects to farms and cities, the river will be too warm for the fish. Water temperatures over 56°F (13.3°C) are deleterious to the salmon, and temperatures over 60°F (15.5°C) are lethal.

Officials with the federal Central Valley Project are now studying ways to change water delivery plans or lower the river-

water temperature to spare the winter-run salmon. The species is protected under the Endangered Species Act.

But many other species of fish are also in danger, and are not yet covered by the Endangered Species Act, says Peter Moyle, professor of wildlife and fisheries biology at the University of California at Davis. Moyle recently completed a survey of all the native fishes in the state and found that of the 113 species, 74 were in need of special protection. Six of those species had become extinct since 1957, and 16 were formally listed as endangered. Most of the damage had been done by water diversions and the introduction of predatory game fish, but for some species the drought could be the final straw. Moyle predicts that with another year of drought the state could add delta smelt, coho salmon and spring-run chinook salmon to the list of species that have disappeared from California waters.

E.S.

FRANCE

in Marin County, north of San Francisco, where conditions are so extreme that the water district has imposed a mandatory water use limit of 50 gallons per person per day. The county ran a pilot desalination plant for three months last autumn, using the process of reverse osmosis to remove salt by forcing seawater through fine membranes.

Voters will decide in November whether to build a \$60-\$120 million permanent plant, rather than building pipelines to import river water from a neighbouring region.

A desalination project about ten times larger, designed to generate 100 million gallons of drinking water a day, is under consideration by a team of Southern California utility companies.

A six-month, \$600,000 study started this month to evaluate the feasibility of constructing the plant across the California border in Mexico. Rather than reverse osmosis, the huge project may use the other common desalination technology, distillation, in which the sea water is boiled and the fresh water condensation collected.

There are those in California who are not content with harnessing the sea and the clouds for fresh water, but who are thinking even bigger — on the scale of rearranging the entire North American continent to equalize the fresh-water supplies which are concentrated in Alaska and Canada. Ideas for ambitious projects have been tossed around for decades, but one of the most overwhelming was revived last year by a group now known as Citizens for Water and Power in North America, Inc. (WAPNA).

Led by Robert Finch, a former lieutenant governor of California, WAPNA proposes to dam three rivers in Alaska and Canada's Yukon Territory, and direct water through a vast chain of reservoirs, dams and trenches into the largest water catchment ever attempted — a 500-mile-long reservoir created from a gorge in the Canadian Rockies — before delivering water to 23 states in the United States as well as regions of Canada and Mexico.

The project would take 30 years to build, planners estimate, so clearly would not ease this drought, but it might relieve the next one. Planners say the project, at an estimated cost of \$300,000 million, would deliver 160 million acre-feet of water per year. (California consumes about 34 million acre-feet per year.) It would also yield 70,000 MW of hydroelectric power, of which 30,000 MW, or about 10 per cent of the current American consumption, would go to the United States.

Various proposals for massive relocations of water have been put forward for years, but nothing close to this magnitude has been successful. Mega-projects are inherently difficult to nurture, given the daunting political and financial hurdles they must surmount, not to mention opposition from environmental groups.

Finch, however, hopes that the current drought may provide him with more sympathetic listeners. **Elizabeth Schaefer**

Budget cuts for research

Paris

FRENCH researchers will have to tighten their belts over the next few years as a result of a significant slowing in the nation's economic growth, made worse by the cost of the Gulf War.

Initially forecast at 2.7 per cent, growth this year is running at 1.5 per cent, mostly because of reductions in rates of value-added tax to bring France in line with its European neighbours.

Last week, the finance minister announced cuts of around FF1,000 million (\$189 million) in the FF48,700 million 1991 civil research budget. But cancelled programmes whose cost was to be spread over several years mean a further FF2,400 million will be lost. Instead of progressing by 6 per cent, as promised, the civil research budget will now be only 0.5 per cent ahead of inflation, currently estimated at 2.5 per cent.

At the ministry for research and technology (MRT) there is a mixture of relief and concern. Denis Plantant, budgetary adviser to research minister Hubert Curien, says that the cuts are "less than we expected initially".

This year, MRT's budget has been cut by FF200 million (0.8 per cent), although when cancelled long-term investments are taken into account, the figure is FF349 million. But, adds Plantant, the effects on the re-

search organizations (such as the Centre National de la Recherche Scientifique, CNRS) will be "not negligible".

These organizations, responsible for most of the nation's basic research, will be especially affected by cancelled long-term programmes, while their budgets for laboratory running costs are also to be reduced by 3 per cent. CNRS alone will lose FF64 million this year (out of a FF11,100 million annual budget), with more than FF102,000 million cut from long-term investments in equipment and construction.

Although the research organizations are to bear the brunt of the new cuts, the government's priority areas — industrial research and the universities — have been largely protected. University research and teaching have not been touched, while spending on industrial research is down FF40 million out of a total budget of FF4,000 million.

France is a major contributor to European technology programmes and to the European Space Agency, but the cuts announced in these sectors are minimal. "France will be able to honour her commitments", says Plantant.

But one official close to the research minister says that French spending on space projects — including the Hermes space shuttle and Ariane 5 launcher — will come under the microscope again in "late spring or early summer".

Peter Coles

BRITISH TECHNOLOGY GROUP

Universities considering a buy-out

London

BRITISH universities, anxious to protect their £13 million annual income from the government-owned British Technology Group (BTG), may try to buy a stake in the company when it is sold into the private sector.

BTG is Britain's largest technology transfer organization. In return for a share in any commercial profits, BTG helps inventors with patent applications, negotiates licence agreements with manufacturing companies, and may even provide funding for research and development (R&D). A government bill to privatize BTG is now passing through parliament (see *Nature* 349, 272; 24 January 1991).

Although welcomed by senior BTG management, the move was attacked by the opposition Labour party, which argues that private ownership would restrict BTG to backing inventions that are likely to give commercial returns in the short term.

The universities receive about £7.5 million a year from BTG in R&D grants, plus some £5.5 million in royalties from BTG-patented inventions. The Committee of Vice-Chancellors and Principals (CVCP) considered the likely impact of the privatiz-

ation at a meeting earlier this month, where several vice-chancellors argued that a buy-out is the best option to protect the universities' long-term financial interest in BTG.

But BTG's price tag, at £35 million, is more than the financially stretched universities can afford. CVCP staff are now investigating the possibility of putting together a consortium to share the cost of a BTG buy-out, making discreet advances to other interested bodies, including large British research foundations.

A university-led buy-out would ease the concerns of university-based inventors, but some resentment over the government's handling of the privatization would remain. Peter Mansfield, from the University of Nottingham, who in the early 1970s developed Magnetic Resonance Imaging, one of the outstanding commercial successes in the BTG portfolio, says that the government and BTG have ignored the views of BTG's inventors, whilst consulting with "all sorts of secondary bodies", such as the CVCP. Mansfield would like to see shares in BTG offered to the investors responsible for "the creative work" behind BTG's commercial success. **Peter Aldhous**

No end of blame on costs

Washington

CONGRESSIONAL staff are investigating financial records at six US universities as part of a widening probe of academic research costs, officials revealed last week.

In the wake of an investigation of accounting practices at Stanford University that found some \$160 million in questionable research costs since 1983, the congressional General Accounting Office (GAO) is preparing to audit the Harvard University Medical School, the Massachusetts Institute of Technology, the University of California at Berkeley, the University of Southern California and the University of Pennsylvania. Johns Hopkins University may also be audited, GAO officials said last week.

The investigators are focusing on 'indirect costs' — the fraction of government research funding that universities use to pay for overhead costs such as utilities, library costs and other expenses that are not directly for research itself (see *Nature* 349, 361; 31 January 1991). In the past decade, indirect costs at most US universities have grown far faster than the 'direct' costs of the research, a situation that has placed academic researchers in competition with their own administrators over scarce research funds.

Harvard University Medical School, for example, has proposed an indirect cost rate of nearly 100 per cent of direct research costs this year. If approved, that would mean that for every federal research dollar the university receives, it would bill the government for another dollar of overhead. Indirect costs at most other private universities range from about 50 to 75 per cent of direct costs.

At a congressional hearing last week, Representative John Dingell (Democrat, Michigan), chairman of the House Energy and Commerce oversight and investigations subcommittee, announced the results of a new GAO probe into accounting practices at Stanford University, which has one of the highest indirect cost rates — 70 per cent.

"It is important to understand that, when a university mischarges or overcharges the government for indirect costs", Dingell said, "that has the effect of diverting federal money directly from other high-priority research projects that could have been funded with the wasted money."

Stanford, which over the past three months has been the target of an expanding investigation that has revealed improper charges for, among other things, a yacht, flowers and an antique fruitwood commode, has received a total of about \$1,800 million in federal research dollars in the past decade. Of that, some \$164–200 million in indirect costs fell under a blanket of special exemptions to normal accounting rules, the GAO investigators found.

"We chose Stanford as the subcommittee's first case study because of a combination of its high overhead rate (it requested 78

per cent this year) and the complaints of the faculty about that rate", Dingell said. After examining Stanford's financial records, he said, investigators found "a story of excess and arrogance, compounded by lax government oversight".

Since the beginning of the investigation late last year, Stanford has agreed to return \$700,000 in questionable billings, has retained a private accounting firm to do an internal audit, accepted an eight per cent reduction in its proposed indirect cost rate and restructured its accounting system to avoid overcharges in the future. The Defense Contract Audit Agency (DCAA), which is responsible for overseeing Stanford's government billing, has recommended that the university's indirect cost rate be cut further, to 52 per cent, and that all the special accounting exemptions be cancelled.

Fred Newton, deputy director of DCAA, testified that, as a result of a review of indirect cost billing at other universities, the California Institute of Technology had also withdrawn \$500,000 in overhead charges.

SPACE RESEARCH

Space Station takes a blow

Washington

THE Space Station, a \$37,000-million project that has been advertised primarily as a research laboratory throughout its eight-year design history, can no longer be justified on scientific grounds, according to a new National Research Council report.

Over the past year, the National Aeronautics and Space Administration (NASA) has been forced by congressional budget pressures to reduce the station's design capabilities and cut costs, which have more than doubled since the project was first proposed. The latest redesign, in response to a congressional directive to trim \$6,000 million from the space station programme, would reduce electric power, crew size and physical laboratory space — at the cost of its research capability, the NRC panel found. "Space Station Freedom, at the present state of redesign, does not meet the basic research requirement of the principal scientific discipline for which it is intended: (1) life sciences research ... and (2) microgravity research and application", the report states.

In particular, the redesigned station will not carry enough crew members to conduct experiments in a reasonable time (the crew was recently halved to four), will not have enough electrical power or computer resources to run experiments and process data, and will not approach the near-weightless environment required for microgravity materials research, NRC said.

The devastating report may provide the ammunition some members of Congress have been looking for to cancel the project,

Dingell's subcommittee, which has jurisdiction over research funded by the National Institutes of Health (NIH) and other federal research agencies, is best known for its investigations of excessive indirect-cost rates among defence contractors.

Research universities are no less prone to accounting abuses, Dingell said. "In the beginning of this investigation, we believed we had the same ingredients for disaster. Unfortunately for the taxpayers, our worst fears appear to be coming true."

Stanford president Donald Kennedy testified at the hearing that accounting errors had allowed portions of his wedding celebration and depreciation on donated silverware to be charged to research accounts. "We have a problem, and we're taking it seriously", he said.

Under increasing pressure to rescue Stanford's tarnished image, Kennedy has recently announced that the university will shift emphasis away from research and towards teaching (see *Nature* 350, 5; 7 March 1991). New programmes, totalling some \$7 million, will offer financial rewards for excellence in teaching rather than research.

Christopher Anderson

says Robert Park, director of the American Physical Society's Washington Office. "I get the feeling that the wolves are smelling blood and starting to circle", he says.

While the research community has increasingly turned against the Space Station as its costs climbed and its potential scientific payoff waned, the NRC report is the strongest and most shocking condemnation yet.

NASA now finds itself in a difficult — and perhaps untenable — situation, with support for the project quickly evaporating almost everywhere outside the aerospace industry. Last year, concern over the impossible number of space walks that would be required to maintain the station forced NASA to schedule a complete redesign, the second major restructuring in less than a year (see *Nature* 344, 367; 1990). (No construction has begun for the project, which is currently scheduled to be in orbit by 1996.)

The current redesign process alone is costing \$2,500 million a year, more than the entire budget for the National Science Foundation, Park notes.

A recent internal report by the White House Office of Science and Technology Policy (OSTP) to Vice-President Dan Quayle, head of the National Space Council, was also critical of the space station's scientific usefulness. But the OSTP report suggested that some relatively minor modifications could make the station usable as a laboratory to study the way humans respond to long space flights, which might serve as at least one much-needed justification for the project.

Christopher Anderson

Black skies or pale fire?

SIR — By igniting more than 500 oil wells, Iraq has inflicted an insult to the atmosphere rivalling nature at its worst. The local effects are as appalling as the global effects are trivial. Plumes of soot and pyrotoxins darken the skies over Kuwait and its neighbours as the incandescent flames illuminate scenes of apocalyptic destruction.

The demolition of the water supply to the oilfields makes even master firefighters such as Red Adair despair of extinguishing the wells in less than a year. But even in the absence of water, the appalling soot clouds that threaten the region can be fought — with air.

For so much smoke can only arise from an insufficiency of fire. It is incomplete combustion that is the problem: a stagnating columnar flow of oil, mixed with air only by the turbulence of its burning, is clearly a worst-case as regards air pollution. Can combustion engineering ameliorate it?

The flow rate of each burning well falls into a range familiar to the combustion engineers charged with designing efficient

thermal power plants fuelled by oil. The 10-megawatt to half-gigawatt flames at the wellheads in Kuwait are burning only 75–90 per cent of the roughly 1.5–45 kg per second of gushing oil that feeds them. Adding to each a few tens to hundreds of cubic metres of air per second could eliminate their oxygen deficit and turn them into soot-free oxidizing flames. Better a host of pillars of blue fire ascending than a horde of sullen sources of baleful incandescence and stygian gloom.

The existing wellhead fires are more than sufficiently energetic to drive air entrainment by both convection and, at their bases, the venturi effect. Installing passive combustors built of refractory materials, vortex generators and scaled-up variations on the theme of the Meeker burner or the oil-furnace combustor-can need not await the full restoration of the water lines. And where advection is needed to promote complete combustion, ducted fans and compressors of quite modest power can generate the requisite volumes of air flow — a thrust reckoned in tonnes.

It is time to direct the arsenal of combustion engineering swiftly to deploy whatever it can to reduce the life- and ecosystem-threatening emissions of these flames. Besides, increasing their air supply can reduce their emissivity, cooling the now incandescent ground adjacent to them, thus preparing the way for those who will follow in the long war of attrition that will eventually see them extinguished.

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Degrees of freedom

SIR — Recent issues of *Nature* have had articles on the funding for science (or the lack thereof). I would like to address a little-known but serious problem regarding the funding of scientists who by choice have not attained a degree.

The requirements for receiving research grants from all organizations are dependent on several factors, none more important than whether the applicant has a doctorate or at least is in an academic setting. There are a few of us who do not have a degree but make significant contributions to a chosen field. Where do they get their funding? The lucky few are associated with some institution that may provide funds for their particular research interests (this, however, does not in any way put them on an equal footing with those who have a PhD). There are also those who are not affiliated with any institution but who are active in their field purely for the love of the subject. Yet these individuals are severely discriminated against when applying for research grants because they do not possess the required academic papers.

With the economic situation as it stands now, the so-called independent scholar will be even more restricted and less productive. There should be a way to recognize these contributions and provide the necessary funds to keep such people going.

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Raman rejoinder

SIR — In the news item on the Raman Research Institute (RRI) in Bangalore (*Nature* 349, 732; 1991) your correspondent has rightly stated that I have not commented publicly but would do so in an appropriate forum. Perhaps it is appropriate to comment in *Nature* if only to clear up distortions, inaccuracies and omissions in the report.

(1) My recommendation to the governing council on Dr C. V. Vishveshwara's contract was not a "sacking" and the abruptness was only apparent. It was certainly not abrupt to the person concerned. Anyone who runs an institute will appreciate the many avenues one goes through in such a case before seeking more drastic remedies. Since things started to go wrong in the mid-1980s, the process has occupied roughly half of Vishveshwara's stay at the RRI.

(2) The international reputation based on his early work was of course the reason why Vishveshwara was invited to set up a school of research in the first place. Your report deals very cursorily with the more relevant

question of what was achieved (or not) subsequently. Any visitor to the rest of the astrophysics group at the institute can confirm that the overall atmosphere has been one of friendly interaction, open discussions, participation in seminars and training and encouragement of students and young people. Many colleagues (all junior to Vishveshwara) have contributed to this atmosphere and their work has been recognized by the best form of peer review — invitations to talk at international meetings. If Vishveshwara had measured up to these standards, there would have been no need for me to make a negative recommendation to the council about his contract.

(3) The case was by no means "all set to be closed" when the deciding authority (the governing council) took it up. Three meetings were held and a committee was appointed which submitted a unanimous report. An important part of this report was the stress on building a new school in relativity and gravitation as the old one did not take off.

(4) No restriction on collaboration or the use of institute facilities existed before, during or after the council resolution. The reference to personal research, which has been twisted to imply the opposite in your report, simply refers to the council's perception confirming my belief that Vishveshwara's earlier role as the nucleus of a group should come to an end. The decision I took to shift his office was a natural consequence of his changed role and has been blown up out of all proportion. The new office is a pleasant one, in the same building where I work. More research activity will shift there in the future as and when the need for space arises.

(5) The fact that the unanimous resolution of the council overruled my earlier recommendation and that the trust further tempered the contract and my executive actions clearly show the system of due process and checks and balances at work. This is very far from the image of the institute's functioning conveyed in your report.

(6) A good part of the report covers opinions of various individuals and is thus selective though factual.

(7) The institute's main funding agency, the Department of Science and Technology (represented on its council) is needlessly criticized as being ostrich-like. In fact, its stand reflects its own independent assessment of the role and limits of a funding agency.

I am confident that RRI will continue to be judged by the quality of its atmosphere and research output and will not be found wanting. My colleagues who have worked to build up and maintain this quality would like nothing better than to be allowed to return to their task, without disturbance from ill-informed outcry.

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Origin and spread of AIDS

SIR — Some people have decided that the origin of AIDS will be forever associated with Africa, hence such unscientific statements as "there is now little doubt human AIDS began in Africa". Their evidence is that "not only is the disease widespread in Central Africa but only in Africa are there monkey species naturally infected with lentiviruses related to human immunodeficiency virus". An African might have written similarly of syphilis in Europe and in the Middle Ages: "There is little doubt that syphilis began in Europe. Not only is the disease widely spread there, but only in Europe it seems are people naturally susceptible to the disease." At that time, there was not a single case of syphilis reported from black Africa, even though there were sailors going to and returning from Africa to Europe, thanks to acquired immunity against syphilis because of widespread yaws. We all know now that syphilis did not begin in Europe.

If members of the Idjwi tribe had practices that would constitute an efficient means of trans-species transmission and could be responsible for the emergence of simian immunodeficiency virus (SIV) infections of man and thus AIDS, why have they only now developed AIDS? Perhaps A. Karpas (*Nature* 348, 578; 1990) would have us believe that they had acquired an immunity to AIDS

until they suddenly lost it in 1959. Sexual practices in East Zaire, in a small circumscribed tribe, led to a suggestion that SIV could have given rise to HIV-2 infection of man in West Africa thousands of miles away. How elastic is Karpas's imagination?

He goes on to contradict himself by stating that "in spite of long association between Europeans and black Africa there is no evidence for the existence of HIV in Europe, the Americas or Arabia during the past century or even the first half of this century, which argues strongly that the widespread HIV infection Africa is a recent event". So what is the relevance of the peculiar sexual practices of the Idjwi tribe except to ridicule black Africa or to excuse the poor sexual performance of Europeans?

"All in all, the epidemiological evidence thus points to the spread of HIV infection from Africa since the Second World War with the widespread introduction of syringes and needles from the West, together with vaccination programmes." Does the author not find it strange that the first case of AIDS was reported in 1959 or that doctors from Europe practising in Africa during the colonial period were so poorly trained that they could not even describe a disease with such a dramatic impact on the population?

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Sinister change

SIR — Is B-DNA really right-handed? Some recent advertisements suggest otherwise. Genosys (*Nature* 14 June 1990), Zymo-Genetics (*Science* 15 June 1990), Applied Biosystems (*Science* 10 August 1990), Oncor (*Science* 28 September 1990), Clontech (*Nature* 4 October 1990), and Eppendorf (*Science* 25 January 1991), all of which claim DNA expertise, indicate that the molecule is left-handed.

Other experts seem to agree. The newly revised eighth edition of the textbook *Principles of Genetics* by Gardner, Simmons and Snustad features on its front cover in vivid green and yellow a giant drawing of a left-handed DNA molecule.

The Center for Advanced Biotechnology and Medicine, a facility jointly administered by Rutgers University and the University of Medicine and Dentistry of New Jersey and called by the Newark *Sunday Star-Ledger* (26 June 1988) the centrepiece of a "\$50-million gamble", confirmed that remark by adopting as its logo a left-handed B-DNA helix. They have had second thoughts, however, and are now putting their money on the right-handed form. Did they switch prematurely?

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No money

SIR — In the correspondence in *Nature* following the news of the financial problems faced by the Medical Research Council and the Science and Engineering Research Council (SERC), there has been a notable absence of comment from the group of research workers at the sharp end of the current cuts — those of us employed on fixed-term grant-funded contracts. This should not be construed as reticence; rather, it reflects the belief that nothing we say is likely to stimulate solutions to the seemingly intractable problem of extracting more money from a government, locked into a recession, with little concept of the importance of maintaining a strong research base in Britain.

Yet as potential members of the future British scientific community, we must spell out our concerns. This may even help to strengthen the arguments (and resolve) of the more altruistic members of this community (such as the pressure group Save British Science, the retired president of the Royal Society, Lord Porter, and the new chairman of the SERC, Sir Mark Richmond) who have all recently advocated increasing the resources available to young scientists.

The feelings of many of us can be quite concisely summarized. When our grant ap-

plications are highly rated for the quality of their science but are not funded because of lack of money, we — like our tenured colleagues — are extremely frustrated. But, unlike them, we may also find ourselves facing the imminent prospect of unemployment.

Over the past few years, it has become increasingly apparent that the brighter undergraduates and postgraduates are discouraged from pursuing careers in British science by the increasingly poor prospects this affords. No doubt the current situation is exacerbating this problem by forcing even established research workers to consider alternative careers — not because they crave greater financial reward (those who embark on an academic career accept that they will receive a moderate salary), but because even those of us who love the challenges that research offers simply cannot survive on a wage and a prayer.

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AZT patents

SIR — The report by Christopher Anderson (*Nature* 349, 93; 1991) is most useful in regard to the political/legal climate in the United States regarding Zidovudine (AZT). But it is deficient regarding the important prior art upon which the question of inventive step has to be judged.

The *Merck Index* gives US Patent 4724232 and German Offenlegungsschrift 3608606, in the names of J.L. Rideout *et al.*, as the origin of protection — yet there is so far no German patent. The origin of the US claim resides in two British applications by the Wellcome Foundation, but they have been withdrawn, as is the case with its British patent application 2181128, withdrawn on 7 April 1988.

A correlation and correction of the essential prior art concerning Zidovudine is given in the box below.

PCT. WO. 7901068, Netherlands application (NLA) 8100177.

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See also US Patents 4780453, 4818538, 4828838, 4833130 and 4837208.

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US astronomers ask for more

David Lindley

A survey of the state of US astronomy and of what is needed in the coming decade may be a useful model for those wishing to present a coherent case for science in general. But there are pitfalls.

ASTRONOMERS and high-energy physicists have earned themselves a curiously mixed reputation among the rest of the US scientific community. They have been remarkably successful over the years in extracting from the federal government money with which to build the large facilities (telescopes and particle accelerators) they undoubtedly need, but their victories have attracted as much resentment as admiration. Those working in other fields, rather than seeing the efforts of astronomers and high-energy physicists as something to emulate, seem to assume instead that the success of 'big science' is directly the cause of their own perceived impoverishment.

Behind this envious reasoning lies a flawed hypothesis: that science funding is a 'zero-sum' game, so that funds that go to one group are necessarily denied to others. This is wrong for two reasons. First, there is no such thing as the US science budget, if that is taken to mean a single pot of money from which all research funds come: an increase for one group does not automatically bring a cut for someone else. Second, the total amount of money spent each year by the US government is an imprecisely known quantity (depending on assumptions about future changes in interest rates, industrial output, the strength of the dollar and so on), and the entire science budget is comparable to the uncertainty: science spending could be doubled or eliminated altogether without making any sensible difference to the US economy.

With even this bare understanding of the US budget process, it should be clear that the way for science to prosper is for researchers to argue the cause of their own discipline, without disparaging others, in the expectation that from these separate efforts collective benefits will come.

Successful series

Those who are not astronomers should therefore not automatically prepare to disdain this week's release of the Bahcall committee's survey on the state of US astronomy and astrophysics as another attempt to put special pleading ahead of the general good of science. This survey, "The Decade of Discovery in Astronomy & Astrophysics", has been produced for the National Academy of Sciences by a committee under the direction of John Bahcall, of the Institute for Advanced Study at Princeton, and is the fourth of a series of surveys appearing at intervals of a decade. The series has been successful in ways that all scientists should ponder.

Astronomers, like high-energy physicists, have turned an apparent disadvantage — the need for large, expensive facilities — into a crucial element of their success. Because the scientific communities in these two areas are relatively small (there are thousands of astronomers and high-energy physicists, tens of thousands of biomedical researchers, hundreds of thousands of chemists) and because even the United States can afford only a handful of large telescopes and particle accelerators, collaboration and cooperative planning are essential. In both areas, the political reality that only one or two expensive projects can be undertaken at one time is so obvious that the need for researchers to present Congress and the president with a number of reasoned priorities, rather than a lengthy shopping list of undifferentiated desires, has rarely been controversial.

But having advantages and exploiting them are two different things. What chiefly seems to rankle with opponents of big science is not that certain areas of science demand expensive facilities, which is undeniable, but that those involved have successfully argued for them. Can it be possible that academic researchers have elbowed their way into Washington, agitated for funds, fought off opponents, won their victories, and then returned, their principles uncompromised, to science?

If there is a compromise, it is simply that astronomers and high-energy physicists have voluntarily renounced the principle, still held inviolable in other disciplines, that they should be free to do all the research they want on a timetable of their own choosing. In choosing to lobby for the Superconducting Super Collider (SSC), for example, physicists have deliberately postponed serious discussion of the alternatives, such as a giant linear collider, for more than a decade. Not every high-energy physicist thinks that the SSC was necessarily the best option, but that disagreement has been kept away from congressional hearing rooms.

Similarly, the predecessors to the Bahcall astronomy survey were able to advance the general cause by deliberately aiming to move forward on specific fronts. The Greenstein report of 1972 had as one of its main recommendations the building of the Very Large Array (VLA) of radiotelescopes in New Mexico; the Field report of 1982 argued for an augmentation of the VLA, as well as for a global network of solar telescopes and a series of satellite observatories. Not all of these projects have won federal backing; many are delayed. But the consequence of

the surveys has been that the general wishes of the astronomical community are crystallized into specific aims, which can be fought for in Washington and, more importantly, fought for again after the inevitable setbacks.

The Bahcall report, in this tradition, gives high priority to a number of observational projects (see table). The single biggest item is the Space Infrared Telescope Facility (SIRTF), a Hubble-class (in terms of cost) observatory that would offer a vast improvement (100,000 photon detectors against 62) over the Infrared Astronomy Satellite, launched in 1983. SIRTF, a 0.9-metre telescope in a 100,000-km orbit, supplied with enough liquid helium to keep its detectors working for five years, will complete the US 'Great Observatories' programme, providing, with the Hubble Space Telescope and the already approved Advanced X-ray Astronomy Facility (AXAF) and Gamma-ray Observatory (GRO), coverage from space across the whole spectrum.

Space versus ground

SIRTF aside, the report recommends against large space-astronomy projects, leaning instead towards an array of smaller satellites designed for specific jobs, at reasonable cost, and without incurring the unwieldiness of organization that led to Hubble's aberrant mirrors. A chapter on the possibilities for lunar astronomy recognizes the potential worth of, say, a huge interferometric array of radio-telescopes tied to a rock-solid base, but shies away from encouraging any large efforts towards turning the Moon into an observing base. The committee is evidently keen to avoid any awe-inspiring but technologically dubious plans whose worst fate would be a hearty political endorsement, leaving astronomers stuck with a giant, open-ended, money-absorbing project whose success they could not guarantee. The report suggests instead that as part of a balanced space-astronomy programme there should be effort to identify small projects which could feasibly be transferred from Earth orbit to lunar base, at first with little or no human intervention, and that only then should larger plans be countenanced.

Despite the obvious attractions of space astronomy, the Bahcall report sternly directs most of its recommendations to diagnosing the health of ground-based astronomy, and recommending treatment.

In many of the proposals, infrared is the wavelength of choice, a decision dictated largely by the huge technical improvement of infrared charge-coupled devices (CCDs) in

recent years. An 8-metre infrared optimized telescope for Hawaii is strongly recommended, as is the Stratospheric Observatory for Infrared Astronomy (SOFIA) — a telescope fitted into the back of a Boeing 747, and designed to replace the aging Kuiper Airborne Observatory. Another longstanding desire of the astronomical community, the construction of an 8-metre telescope in the Southern Hemisphere, also makes it onto the list of strongly recommended projects. And there is renewed support for GONG (Global Oscillations Network Group), a system of small, largely automatic solar telescopes distributed around the globe so as to record solar surface oscillations continuously. This is a project whose results so far have revolutionized our understanding of the solar interior, but which has constantly struggled for funds.

RECOMMENDED EQUIPMENT INITIATIVES COSTS

Initiative	Decade cost (\$ million)
Large programmes	
Space Infrared Telescope Facility (SIRTF)	1,300
Infrared-optimized 8-m telescope	80
Millimeter Array (MMA)	115
Southern 8-m telescope	55
Subtotal for large programs	1,550
Moderate programmes	
Adaptive optics	35
Dedicated spacecraft for FUSE	70
Stratospheric Observatory for	
Far-Infrared Astronomy (SOFIA)	230
Delta-class Explorer acceleration	400
Optical and infrared interferometers	45
Several shared 4-m telescopes	30
Astrometric Interferometry Mission (ALM)	250
Cosmic-ray telescope (Fly's Eye)	15
Large Earth-based Solar Telescope (LEST)	15
VLA extension	32
International collaborations on space instruments	100
Subtotal for moderate programmes	1,222
Subtotal for small programmes	251
DECADE TOTAL	3,023

In drawing up such a list, the Bahcall report follows the pattern of the Greenstein and Field surveys, but to see the survey as merely a list of astronomers' wishes is to miss its main thrust and its most innovative features. The compilers of the survey were faced with something of a problem: apart from the lamentable difficulties with the Hubble Space Telescope, astronomy appears to be doing quite nicely. The first stage of the segmented-mirror Keck Telescope in Hawaii is complete, the Mount Graham observatory in Arizona seems to be on its way at last, having overcome 200 squirrels, Magellan is taking pictures of Venus, Galileo is on its way to Jupiter and the Astro-1 satellite, despite operational problems, turned out a remarkable quantity of observations in the ultra-violet. So what more do astronomers need?

This rosy picture is misleading. Last year was a good one for astronomy in space, and

more launches are imminent, but space projects take years from start to launch, and the Bahcall report is addressed to the coming decade. For space-based astronomy, the pump needs to be primed.

As for ground-based astronomy, it has emerged that most of the well-known projects in the offing are privately financed in one way or another, and will not be national facilities as are the Kitt Peak Observatory, the Cerro Tololo Inter-American Observatory in Chile, and the Very Large Array. The very fact that universities are getting together with private foundations to build telescopes, as in the case of Keck, is indicative of the deterioration at the national observatories. At Kitt Peak, indeed, people have been gloomy for some time. Some of the smaller telescopes have been or are likely to be shut down, and funds for visiting astronomers

and routine maintenance are scarce.

The strongest recommendation of the Bahcall report, therefore, is not for the building of new facilities but for the upkeep of existing ones. Detectors need to be upgraded, computer systems augmented, walls painted; grants for astronomers to visit the national observatories are skimpy; theoretical astrophysicists, whose interpretation of observations is as essential as the data themselves, scrape up bits of money from here and there. For all these problems, the need is not for new initiatives or grand plans, simply for more money.

This is a plain enough message, backed up with quantitative arguments, but it is also an unexciting one, not readily distinguished from the cries heard from all the other groups of scientists clamouring for attention in Washington.

It is not, in fact, a complaint unique to science at all. As the Gulf War subsides and attention slowly returns to domestic matters, underground tremblings of discontent with the "crumbling infrastructure" of the United States resume their rumblings through Congress. Bridges are falling, sewers collapsing, inner cities decaying: no one denies the problems, but no one, least of all the President of the United States, has taken responsibility for fixing them.

If astronomers are to add their voices to this general lamentation to any useful effect, they must say something distinctive. The Bahcall report tries: before starting their deliberations, panel members went to Washington to ask people in Congress, in the White House and at the Office of Management and Budget what they wanted to know (a startling innovation that other scientists would do well to follow).

As a result, the report contains a section

on astronomy and computing, arguing for a nationwide system, overseen by the National Science Foundation, for data archiving, and endorsing efforts to build a high-speed national computer communications network. The idea of a national computer network has a good deal of political support already, so astronomers can only win by adding their weight to the proposal. But other suggestions may not be so fruitful. The report argues for increasing the status of theoretical astrophysics by supporting it (within NASA) in approximate proportion to support for observational programmes, an appealing idea which, given NASA's ultimate responsibility to judge individual proposals on their merits, seems impracticable; it also suggests the creation of a separate theory programme within the NSF, something that is sure to increase bureaucracy but may or may not yield more research grants.

Education

The report also devotes many pages to the possibilities of using the expertise of astronomers to improve the supposedly ailing US education system, by arranging summer programmes and internships for students and teachers alike. One can hardly criticize scientists who publicly declare that it is in their interests and that of the United States to come to the aid of the high schools, but there are dangers in trying to turn this sort of neighbourliness into a formally declared policy.

Educational policy in the United States is a battlefield between federal and local administrators, and as in all countries education is perceived as a way to turn the nation's youth not simply into educated citizens but into right-thinking ones: witness the incessant battles over prescribed reading lists, religion and the like. In this minefield, the purest of motives and most neutral of politics are little protection. Astronomers who wish to visit their local high schools and do a bit of teaching will find themselves welcome, but if astronomers *en masse* start agitating for a place on school boards and policy-making bodies, they may find their enthusiasm rapidly worn down.

On the other hand, scientists who want to lobby for their brand of research are likely to get their feet dirty one way or another. It is quite some time since talking of unlocking the secrets of the Universe and wearing mismatched clothes were enough to win support from politicians enamoured of science in all its manifestations.

Harder questions are being asked in Congress these days, and money as well as respect goes to those with the best answers. The Bahcall report is an attempt to promote astronomy not simply by recounting its many wonders and successes, but by setting out a case for astronomy in an economically and politically constrained world. Scientists of all kinds would do well to chart its progress. □

David Lindley is Associate Editor of *Nature*.

Weak equivalence in the balance

If the weight, but not the inertial mass, of an antiproton should differ from that of a proton, there would be important consequences for the theories of relativity and of particles. But informed opinion is sceptical of the possibility.

THE idea that the weights of protons and antiprotons may differ from each other is not nearly the arcane speculation it might seem. Indeed, it is not just a speculation, but the focus of some considerable interest among experimentalists. For one thing, there is a proposal directly to measure the difference of the weight of the two particles now being implemented at the European high-energy physics laboratory, CERN, at Geneva.

The neatness of the experiment is almost a sufficient justification of it, which may (as will emerge) be just as well. The plan is to cool antiprotons from the LEAR storage ring to a few degrees kelvin, and then to launch them vertically upwards in a drift tube and to measure the time it takes for them to reach a predetermined height. There is even talk of carrying out an essentially static measurement of the weight (or gravitational attraction) by letting atoms of anti-hydrogen (a positron bound to an antiproton, yet to be manufactured) fall freely under gravity, which would avoid the trouble to be expected from stray electric fields in a measurement of free charged particles.

But why? The inertial masses of a particle and its antiparticle should be identical, so that any reasonable theory of gravitation, such as general relativity, should enjoin equal weights: weigh one, and you have weighed the other. But that is not quite the end of the story, at least if hopes of winning a quantum theory of gravitation are taken seriously (as they must be if there is ever to be a unified theory of all the forces between particles). In principle, at least, quantum gravity could allow the weights of the proton and antiproton to differ.

But how? E. D. Adelberger, B. R. Heckel, C. W. Stubbs and Y. Su of the Washington University, St Louis, give a cogent explanation in the course of arguing that the observations already made and recorded provide as good a test of the reality of a weight difference as the planned experiments are likely to provide (*Phys. Rev. Lett.* **66**, 850; 18 February 1991).

If the general theory of relativity is ever consistently quantized along the lines of electrodynamics, for example, the gravitational forces between different particles will no doubt be mediated by other particles just as the forces of electrodynamics are mediated by photons, which are massless particles whose spin is 1. Indeed, there is already a name for these particles, which are called 'gravitons', presumably (like photons) with no mass. Moreover, the spin of the graviton

must be 2, or twice that of the photon, which ensures that gravitational forces are always attractive.

The snag is that, in quantum mechanics, hardly any state is ever completely pure in the strict sense of that word; in this case, the spin-2 gravitons may well coexist with gravitons of spin 1 and spin 0. And then the universally attractive forces of gravitation would be mixed with repulsive forces mediated by the spin-1 gravitons and also with attractive forces arising from the spin-0 particles.

Recollections of the excitement over the 'fifth force' of a few years back, far from being out of place, are accurately pertinent, and for two reasons. First, the fifth force was also supposed to be mediated by particles of integral spin (or bosons). Second, the spate of experimental measurements provoked by that excitement may have tightly constrained the extent to which materials of different chemical composition are differently affected, but none of them has excluded the possibility that particles and antiparticles may be differently affected by long-range forces resembling those of gravitation.

There is an even simpler way of putting the conundrum. It is fair to hold that the greatest of Newton's discoveries was the implication that inertial mass (defined as the ratio of the force on an object to the acceleration it produces) and gravitational mass are strictly equivalent. The relativistic equivalent of that principle, called the 'weak equivalence principle', is that one must also take account of whatever internal energy an object may have by the familiar $E = mc^2$ rule. So differences between the weight of protons and antiprotons would, on the face of things, imply violations of the weak equivalence principle.

In the same issue of *Physical Review Letters*, Richard J. Hughes and Michael H. Holzschneider, from the Los Alamos National Laboratory, argue that 'CPT' symmetry, which is an article of many faiths, may alternatively be violated (**66**, 854; 18 February 1991). In this rubric, C stands for the operation of charge conjugation, which replaces each particle by its antiparticle (whenever possible), P stands for the parity operation (which is space inversion) and T stands for time inversion. Or, turning the argument around, if CPT symmetry is exact, and if antiprotons weigh differently from protons, the weak equivalence principle must be violated.

What do existing data say? Adelberger *et al.* argue that data such as those accumulated

during the hunt for the fifth force (including a more generally directed measurement of their own intended to measure the differences (if any) of the gravitational acceleration of pairs of materials such as beryllium and copper) exclude the possibility that quantum gravity may give antiprotons and protons different weights or, more accurately, they say that any gravitational interaction caused by spin-1 gravitons is unlikely to amount to more than one part in 100 million of conventional gravity.

They go on to exclude the theoretical possibility that the effects of spin-1 and spin-0 gravitons may cancel in protons, but not in antiprotons, by insisting that cancellation would not be possible for all pairs of materials and for all the physical dimensions (from a metre to some millions of kilometres) over which the equivalence principle has been verified.

Hughes and Holzschneider are similarly despondent at the prospects of finding that antiprotons weigh differently from protons. For one thing, they note, there have already been measurements of the cyclotron frequencies of protons and antiprotons that effectively restrict the difference of their weight to less than one part in 1,000, a tighter limit than likely to be attained in the first version of the experiment planned at CERN. They go on to argue that more stringent limits will quickly be possible as the effectiveness of electromagnetic traps for single particles improves.

So, on the face of things, the planned experiments are unlikely to yield surprises when the first results emerge, perhaps two years from now. Yet that is not a reason why they should be abandoned. For one thing, they are neat. For another, even if other than a null result would be a violation of the weak principle of equivalence which has been amply verified in other circumstances, there are the strongest reasons why principles of such importance should be tested in circumstances as varied as they can be.

Hughes and Holzschneider raise a further question of literally cosmic significance: if gravitons are not strictly massless, but have a small mass (as is canvassed for some neutrinos), the range of gravitational forces will not be infinite, but may still be of galactic or cluster scale, suggesting that the most productive experiments will be those comparing cyclotron frequencies, say, here and at the distant reaches of the Universe. There is a challenge for the experimenters.

John Maddox

Revealing corrosion pits

G. Timothy Burstein

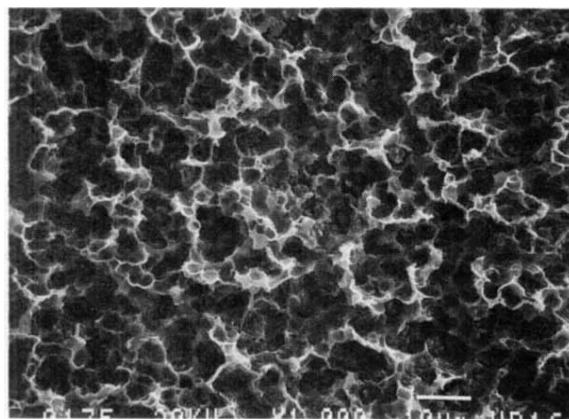
It is particularly refreshing to those in the field to find new theories injected into current knowledge of metal corrosion. This is the impression laid by the stimulating article of Williams *et al.* on page 216 of this issue¹, in their novel description of the phenomenon of pitting corrosion of binary alloys. The ideas involved are really very simple; they call on the theory of percolation through clusters of atoms to explain why pitting corrosion in binary alloys, such as Fe/Cr stainless steels, can be nucleated. Conventional ideas on the nucleation of pitting corrosion require a specific act of aggression on the surface oxide film, which normally passivates engineering metals, by a specific component of the liquid environment. Such ideas include concepts of inherently weak spots in the film, oxide-film rupture by vacancy condensation underneath the film, and dissolution of the film through complexing by aggressive ions. The proposal of Williams *et al.* by-passes all that by invoking the exposure of clusters of iron atoms (in a stainless steel matrix) during the normal passive corrosion state of the alloy. Once exposed, the iron cluster dissolves away, and a pit is born.

Corrosion is these days reckoned² to cost industrialized countries some 3–5 per cent of the gross national product. Pitting corrosion is one component of this cost. It is a particularly insidious and dangerous form of metal corrosion, because it often goes unseen and unnoticed until failure occurs. Telltale signs of corrosion of metals in service, such as the formation of rust on steel or the appearance of blue water from copper plumbing, are frequently absent when pitting corrosion commences, and may remain absent until failure. The reason is that most of the metal is passive; only on a small fraction of the metal surface does pitting corrosion take place.

Most engineering alloys are used in a state of passivity: the corrosion rate is very low because of an intervening oxide film caused by reaction of the metal with the environment. This film provides a powerful barrier towards ionic migration, thus retarding the rate at which metal ions enter the solution phase, despite what may be a very high driving force towards corrosion. The suggestion by Williams *et al.* that this chromium-rich oxide film on stainless steels may be essentially a monolayer thick is intriguing. Passivating films are known to be very thin, particularly on stainless steels, and there are hints from past work³ that the limiting stable film thickness may indeed be only one monolayer, although the thickness is usually considered⁴ to be a function of the electrode potential of the material, to account for the observation that the passive current density is independent of potential. The idea comes perilously close to the adsorbed monolayer proposed by Uhlig and King⁵; only the

identity of the monolayer is different.

Pitting corrosion can take place when this passive state is broken down locally under the influence of certain aggressive ions in the environment. The most commonly encountered of these is chloride, as found for example in sea-water, although other ions such as bromide, iodide and sulphide also



Electron micrograph of pitting corrosion deliberately induced into aluminium to increase its specific surface area as a step in the preparation of printers' lithographic plates. Individual pits grow at a rate of about $3\mu\text{m s}^{-1}$. Scale bar, $10\mu\text{m}$.

cause pitting. During pitting corrosion, most of the metal surface remains passive, with a measurable low corrosion rate; typically this could be some picometres per second or even less. In isolated areas, however, the development of pits, microscopic in the first stages, leads to component failure by perforation. Pits are also thought to act as potential sites for nucleation of stress-corrosion cracks. It has confounded many researchers for many years why a pit develops at some point *x* on the surface but not at another point *y* even though the two sites have no apparent distinguishing features. The model of Williams *et al.* shows that the sites are not identical at all; *x* simply carries a larger iron cluster than *y*.

Noted aspects of a developing pit are a high chloride concentration and high acidity within the occluded region, far higher than in the bulk environment⁶, and a fast rate of propagation. The acidity is caused by hydrolysis of oxidation products within the pit, and chloride ions migrate into the pit to maintain charge neutrality. The rate of propagation (which may be measured in hundreds of nanometres or even micrometres per second) itself maintains the concentration differences between the corroding solution within the pit and that without. These three well-known properties interplay with one another, so that once growing, the propagation of a corrosion pit is self-sustaining (sometimes called autocatalytic). Many incipient pits, that is, pits which nucleate, do not achieve the conditions required for self-sustenance, and die through so-called repassivation by regrowth of the passivating

oxide film. The explanation given by Williams *et al.* is again very simple: only if the iron cluster is big enough will the incipient pit survive. If it is too small, the pit dies. The susceptibility of a specific alloy to pitting corrosion, and the sporadicness of pitting events, then depend on the size, geometry and distribution of iron clusters. The known fact that stainless steels of increasing chromium content have increasing resistance to pitting corrosion also has an inherent response in the model: a higher chromium content implies a greater paucity of iron clusters sufficiently large to sustain a pit.

The model also accounts for the induction period, the period required to form the pit. This would become the time taken for the surface corroding at the passive corrosion rate to find a sufficiently large iron cluster. Undeniably, it all seems to fit.

There are many questions that the new model raises. For example, the presence of relatively small amounts of molybdenum alloyed in stainless steels gives a significant improvement in resistance to pitting, a phenomenon known to act synergistically with the effects of chromium itself. This

phenomenon too, has accepted explanations, such as the fact that the molybdate anion is a known corrosion inhibitor. But one must now query whether the effect of molybdenum lies in its possible interactions with iron clusters. For example, is it reasonable that molybdenum may tend to aggregate into iron-rich clusters, or conversely, act to disperse them? Indeed, one can now query such possible interactions for all component elements in stainless steels. Other intriguing questions also require addressing. What is the critical size (and geometry) of a cluster required to cause pitting corrosion and how does this relate to controllable parameters, such as the chloride concentration? What is the nature of the electrolyte, in such a minute occluded region where the dimensions are barely larger than twice that of the electrical double layer?

Not that all can be answered through the new theory. Pitting corrosion driven by chloride ions also occurs on many pure metals, such as iron, nickel, copper and aluminium where such clustering is irrelevant, as well as on alloys of small minor-element content. Pit nucleation here must continue to be explained by one of the older ideas. This then raises the bogie: is it necessary, or indeed

- Williams, D. E., Newman, R. C., Song, Q. & Kelly, R. G. *Nature* **350**, 216–219 (1991).
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even sufficient, to invoke two mechanisms for one phenomenon, that of pit nucleation? Other effects too, such as those of surface roughness, appear to be poorly amenable to the new analysis. Because of the microscopic nature of freshly nucleated pits, direct experimental evidence to support this particu-

lar model (or any other) will be difficult to achieve; equally, the sheer logic of its framework is difficult to deny. □

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CELL BIOLOGY

Probing the plant cytoskeleton

Clive Lloyd

WHEREAS the vigorous movements of animal cells have always hinted at the cytoskeletal turnover now known to occur beneath the surface, the impenetrability of the plant cell wall has blocked investigation of how cytoskeletal dynamics might be involved in the more subtle processes that shape immobile cells. On page 238 of the issue¹, Asada *et al.* describe the incorporation of fluorescent brain tubulin into microtubules of the cytokinetic apparatus of tobacco cells, thereby adding to the strategies for overcoming the barrier of the cell wall. Success in this area will be especially important in understanding the morphogenesis of higher plants, in which microtubules play such a prominent role. A better knowledge of dynamics would indicate how four microtubule arrays succeed one another during the cell cycle; where the microtubules are nucleated; how they turn over; and how the cortical microtubules of interphase cells shift their alignment in response to growth regulatory substances and environmental signals. Such advances would strengthen substantially the weak links between plant cell biology, physiology and development.

All of the recent studies centre on the cytokinetic apparatus — the phragmoplast — which seems to emerge out of the anaphase spindle and forms two circular 'picket fences'

of microtubules which oppose one other at the former spindle equator. Vesicles containing cell-plate material are brought to the mid-line where a callosic disk forms on the equator. As the circumference of this disk increases, its boundary of phragmoplast microtubules moves out centrifugally until the plate fuses with the mother wall. The main questions to be answered are where and how the phragmoplast microtubules originate, and whether they are stable or dynamic during outgrowth of the phragmoplast.

In the tobacco cell system described by Asada *et al.*¹, glycerination is used to arrest cytokinetic progression, and so it is not certain how the observations bear on cytokinesis *in vivo*. But they do indicate potential dynamic activity within the phragmoplast microtubules. The fluorescent tubulin is incorporated, initially, as a thin band at the equator, a result in accord with hook decoration studies² which have shown that that is where the fast-growing (plus) ends of the two sets of phragmoplast microtubules interdigitate at the mid-line. With time, the fluorescent band in the microinjected tobacco cells increased in width. After microinjecting for a second time with unlabelled tubulin, the equatorial fluorescent band split in two and the two fluorescent rings moved away from the equator. This is interpreted by Asada *et al.* as

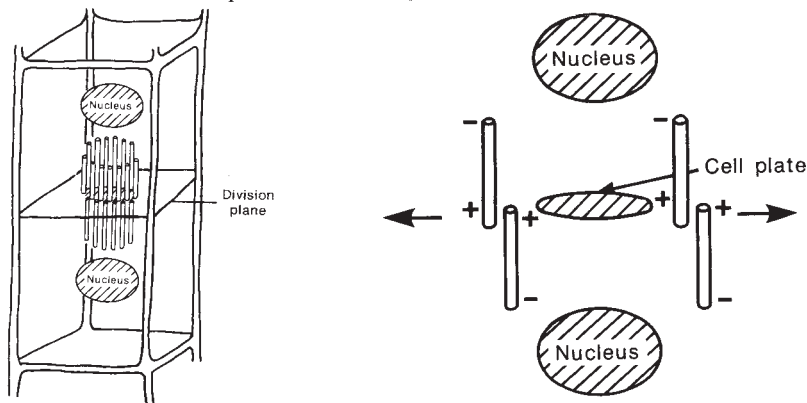
a reflection of tubulin incorporation at the plus ends with concomitant translocation of microtubules away from the equator towards the minus ends; that is, the area of overlap between oppositely directed microtubules is minimized by their sliding apart, causing incorporated tubulin to move away from the mid-line, towards the former poles. Movement is inhibited by the non-hydrolysable nucleotide analogues, AMP-PNP and GMP-PNP, but is induced by GTP and to a lesser extent by ATP.

Microtubule motors have not yet been identified in plants but this work suggests that GTP-splitting motors may be responsible for moving microtubules away from the cytokinetic mid-line. Movement of fluorescence away from the mid-line seems only to occur when unlabelled tubulin is added, and so the implications of these observations for living cells under steady-state conditions are not clear, particularly because taxol is added to stabilize polymerized microtubules. The idea of there being microtubule motors in the phragmoplast is interesting, although the possibility that tubulin subunits added at the plus ends actually flow to the minus ends by flux, as shown for kinetochore microtubules by Mitchison³, might also explain some of the observations.

What of other work in this area? Zhang *et al.*⁴ succeeded in microinjecting living *Tradescantia* stamen hairs with fluorescent brain tubulin, and although cortical microtubules and the preprophase band were difficult to observe, it was possible to follow labelled cells from before mitosis through to cytokinesis. The implication here is that a pool of tubulin subunits is shared by at least the spindle and phragmoplast. Zhang *et al.* found that during late anaphase the fluorescence became more intense in the interzone region, concentrating to form an elongated bundle in the middle of the cell, parallel to the spindle axis. With time this bundle shortened and then began to expand laterally, forming the phragmoplast. Throughout centrifugal outgrowth of the phragmoplast, fluorescence diminished in its centre but increased at its advancing edge.

The evolution of phragmoplast fluorescence out of the interzone of the anaphase spindle implies that there is a continuity between the two structures, consistent with the fact that both share the same polarity in *Haemanthus* endosperm cells². But it is not yet clear whether the phragmoplast is a naturally thrifty way of re-using spindle microtubules or whether the cytokinetic microtubules are newly synthesized from nucleating material in the interzone. The drawback of the first explanation is that it does not account for how phragmoplasts develop between non-sister nuclei (as in cellularization of syncytia) which were never separated by a spindle, but neither is it established to universal agreement where the microtubule nucleating material is located.

Taking another approach, Vantard *et al.*⁵ lysed *Haemanthus* endosperm cells to incor-



Cytokinesis in the plant cell. The phragmoplast microtubules form two circular cages, the ends of which interdigitate at the mid-line. Actin filaments are also found in the phragmoplast, as are abundant vesicles which fuse at the mid-line to form a callose-rich disk (the cell plate). The phragmoplast ring becomes wider as the cell plate grows, until it completes cytoplasmic division by fusing with the mother wall. This centrifugal cytokinesis is to be contrasted with the more familiar purse-string cytokinesis of animal cells. Studies² have established that the fast-growing (plus) ends interdigitate with the slow-growing (minus) ends directed towards the former spindle poles. Phragmoplast microtubules share the same polarity as the half spindles, implying a continuity between mitotic and cytokinetic apparatus, yet phragmoplasts can separate syncytial nuclei which were non-sisters. Studies indicate that tubulin is incorporated at the mid-line, although subsequent motion might be due to a variety of mechanisms.

porate *Paramecium* axonemal tubulin. After fixation, the exogenous tubulin could be located, using an axonemal tubulin-specific antibody, against the background of endogenous microtubules labelled with a more general probe. In this way, axonemal tubulin was seen at late anaphase to be incorporated as a thin equatorial ring. Double immunofluorescence showed that incorporation occurred at the ends of existing endogenous microtubules. Later, in telophase when spindle pole microtubules were less abundant, there was a more extensive incorporation of exogenous tubulin throughout the phragmoplast. Again, initial labelling of a discrete equatorial ring, followed by increased labelling throughout the breadth of phragmoplast microtubules, indicates some special activity of the interzonal region.

Complementary studies⁶ on the microinjection of fluorescent phalloidin into *Haemaphysalis* endosperm cells confirm the special nature of this region in cytoskeletal terms. Label injected at the beginning of mitosis is incorporated into actin filaments that surround the nucleus or run within the spindle, parallel to microtubule fibres. No label is incorporated into the phragmoplast, although this structure can be labelled by a second microinjection at late anaphase or telophase. This implies that the phragmoplast 'wreath' of F-actin filaments is a newly polymerized structure rather than being formed from redistribution of the filaments stretched between the poles at metaphase. An incidental aspect of such studies from Lambert's laboratory, which indicates that actin antagonists impede chromosome separation, is the challenging possibility that F-actin might play some part in plant mitosis.

It will be important to establish whether there is continuity between spindle and phragmoplast microtubules or whether there is a new phase of nucleation at the mid-zone. The possibility of microtubule motors (as a cause of microtubule movement within the phragmoplast) is enticing, although it is too early to say whether or not tubulin incorporated at the interdigitating plus ends might move through microtubules by flux, or whether minus-end assembly is involved. In more general terms, the encouraging message of these recent papers is that incorporation of probes into the plant cytoskeleton is feasible, and that it is now possible to study turnover and movement in living plant cells. Given the centrality of microtubules to plant morphogenesis we can expect exciting developments as such studies are extended to interphase and pre-mitotic cells. □

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Seven genes for three diseases

Richard D. Wood

NEW twists to the connection between DNA repair and cancer emerged at a meeting last month*, with reports that the relationship between two inherited repair disorders having very different cancer risks is much closer than previously thought. Moreover, the relationship of these syndromes to a third unusual condition was strengthened.

People suffering from the skin tumour-prone disease xeroderma pigmentosum (XP) are highly susceptible to sunlight, and their cells display deficiencies in the excision from DNA of damage caused by ultraviolet light. The XP complementation groups A to G represent different genes in the excision-repair pathway, and mutations in any one can give rise to some form of the disease. Now it seems that Cockayne's syndrome (CS), which was previously considered to be quite distinct, may arise from mutations in different components of the XP-repair system. And another disease, trichothiodystrophy, which causes brittle hair, shows tight genetic linkage with an XP gene. Studies of the particular mutations in the seven or more genes that can lead to these three conditions may provide a model for other pathogenic conditions arising from alterations in proteins that cooperate to form a functional complex. Unexpectedly, the new data have made it clear that some individuals harbour a severe DNA-repair deficiency without an increased risk of skin cancer.

In the more common forms of Cockayne's syndrome, patients show sensitivity to sunlight, but the freckling, dermatosis and cancerous lesions of xeroderma pigmentosum are absent. Instead, other distinctive physical and neurological defects are displayed. Cells from individuals suffering from Cockayne's syndrome seem to have a subtle defect in excision repair that is restricted to actively transcribed genes¹. One connection between the two diseases was already known: the only well-characterized individual from XP group B had symptoms of both diseases (see the discussion in News and Views last year²).

Recently, at least six more patients with both xeroderma pigmentosum and Cockayne's syndrome have been identified. Two have been assigned to XP group B, but, surprisingly, three fall into group G and one into group D (D. Bootsma, Erasmus University, Rotterdam; A. Lehmann, University of Sussex). Another patient with both diseases was previously allocated to the spurious group H, but has now been assigned to group D (independent evidence from Bootsma; R. Johnson, University of Cambridge; M. Stefanini, CNR, Pavia). But not all patients with CS show symptoms of XP, nor do all XP sufferers show signs of CS. It appears that allelic differences between mutations in this family of genes can lead to one disease, or the other, or some combination of both.

The hallmarks of the third disease, trichothiodystrophy³, are brittle, sulphur-deficient hair, mental and physical retardation, abnormal nail growth and scaly skin. About half of 21 patients that have been carefully studied are photosensitive, and they show defects in DNA repair that correspond to XP group D by complementation analysis (Stefanini). Others afflicted with trichothiodystrophy clearly do not have xeroderma pigmentosum, and so the intriguing relationship is most easily explained by there being a very close genetic linkage of two genes. No doubt a more precise biochemical definition of the altered sulphur metabolism in trichothiodystrophy would help in resolving this issue.

Neither the XP-D/trichothiodystrophy patients nor the two new XP-B patients (in their late thirties) have skin tumours. This sort of observation is directing attention to immunological surveillance systems in xeroderma pigmentosum, and their possible role in the aetiology of solar tumorigenesis in the disease^{4,5}. Provocatively, six out of the seven patients with xeroderma pigmentosum examined so far in one study showed about one-third of normal natural killer-cell activity; the exception was an individual who had experienced multiple, self-healing melanomas over the course of several decades, suggesting the existence of an especially efficient anti-tumour defence system in this patient. People with Cockayne's syndrome and trichothiodystrophy but without XP symptoms or tumours had normal immune function (P. Norris, St Thomas's Hospital, London). Other factors, including pigmentation, certainly can influence the occurrence of skin cancers. For example, such carcinomas are very rare in Japan, even in individuals suffering from xeroderma pigmentosum who have severe repair defects (H. Takebe, Kyoto University).

The existence of people with mild xeroderma pigmentosum and a marked DNA-repair deficiency but no tumours might suggest that the proportion of asymptomatic carriers of DNA-repair alterations is larger than previously suspected. If this is indeed the case, our views of the effects of environmental mutagens and carcinogens on the general population could require substantial reassessment. □

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*A New Look at Excision Repair Disorders, Eastbourne, UK, 27 February–1 March 1991.

Synthesis with a strong hand

John M. Brown

NINETEEN ninety one has opened with a rash of reports¹⁻⁴ of new procedures for catalytic asymmetric synthesis. Together they show that organic chemistry is moving closer to one of its principal goals — the ability to make new carbon-carbon bonds with complete control of three-dimensional structure under very mild conditions.

Saturated carbon atoms, which constitute the backbone of most organic compounds, are connected to their neighbours through a tetrahedral arrangement of bonds. If the four bonds are to different groups, that carbon atom provides an asymmetric centre and can exist in two mirror-image forms, termed enantiomers. In most syntheses it is crucial to produce the correct enantiomer, because the wrong one will have quite different biological properties; furthermore, a molecule with more than one asymmetric centre will exist as different stereoisomers which have different physical properties. Thus controlled asymmetric synthesis is central to the aim of creating three-dimensional structures.

Tetrahedral centres are most frequently synthesized from trigonal precursors, as the example in Fig. 1 demonstrates. The problem is to discriminate between attack at the upper and lower faces of the carbonyl group, as drawn. In the natural world, this is effected by the asymmetric environment of an enzyme active site which binds the complete molecule in such a way as to ensure that the reagent can only approach from one face. The chemist cannot (yet) design reagents which can bind so selectively and comprehensively to their reactants and so uses the more limited device of specific binding of the reagent at the reaction centre.

To make a further comparison with the natural world, enzymes act in a highly catalytic manner, one molecule of enzyme turning over thousands of molecules of reactant. Traditional chemical reagents generally react on a one-to-one basis, but efficient catalytic asymmetric syntheses, as powerful as enzymes, are being discovered on an almost weekly basis.

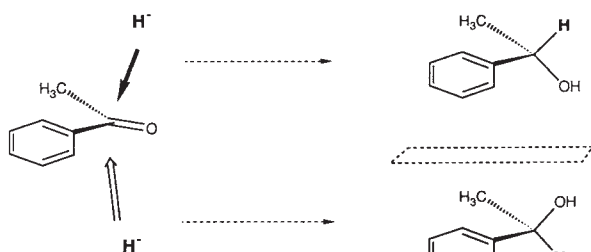


FIG. 1 Making a new asymmetric centre by addition to a double bond.

One of the highlights among the recent reports comes from Schmidt and Seebach¹, who have found an interesting new catalyst for the transfer of an ethyl group from diethyl zinc to aldehydes. This is a well-established

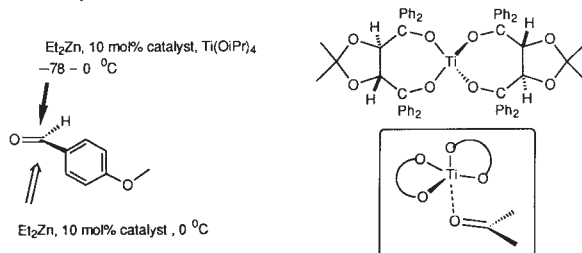


FIG. 2 Titanium complexes for control of the chirality of diethylzinc addition to carbonyl compounds.

asymmetric synthesis, which has been known since the mid-1980s and was broadly discussed in an earlier *Nature* article⁵. The new contribution demonstrates that the novel titanium complex shown in Fig. 2 is an effective catalyst for the reaction. The acidic titanium centre is necessary since it binds to the carbonyl oxygen of the aldehyde and enhances its reactivity. The rigid molecular framework of the titanium complex, with its strongly twisted conformation, acts as a chiral assembly wherein one face of the carbonyl group is accessible to the alkylzinc reagent, and the other is inaccessible. Under the conditions quoted, 91 per cent of one enantiomer and 9 per cent of its mirror-image isomer are produced in a reaction which is catalytic in titanium, representing a satisfactory, but not spectacular, asymmetric synthesis. At rather higher concentrations of catalyst, the selectivity is better.

Apart from further studies which should permit a detailed molecular description of the geometry of the reacting complex, that would be the end of the matter. But further experimentation provides startling complications. When a stoichiometric amount of a simple titanium complex, $\text{Ti}(\text{OiPr})_4$, is added at very low temperature with the catalyst and aldehyde, and allowed to warm up slowly to room temperature, then 99 per cent

of one enantiomer is produced, but it is the opposite enantiomer to the one seen in the earlier experiment — so both hands of product can be prepared using a single hand of catalyst. Obviously the second titanium complex is associating with the aldehyde, the chiral catalyst or both in some as yet unspecified way. This enticing but infuriating observation demonstrates the central fea-

ture of much current work — it is possible to make startling discoveries but our depth of understanding of them is insufficient to provide real predictive power.

Another set of results is more encouraging to those who need less complicated successes. The attractiveness of particular catalysts is enhanced if they are both efficient and readily available, and on this basis the semicorrins of Fig. 3 are very hard to beat. They are easily prepared from chiral amino-alcohols (ex amino acids), and since the first applications to asymmetric synthesis by Pfaltz and colleagues² they have rapidly gained in popularity. Two Harvard groups have now made fresh advances. From Evans's laboratory³ has come the best current method for catalytic asymmetric synthesis of cyclopropanes, a process central to the preparation of environmentally friendly chrysanthemic acid-based insecticides. And Corey and coworkers⁴ have demonstrated that iron-semicorrin complexes are good catalysts for the asymmetric Diels-Alder reaction, the most useful synthetic method for construction of polycyclic compounds. Making the reaction both asymmetric and catalytic is a considerable challenge, and

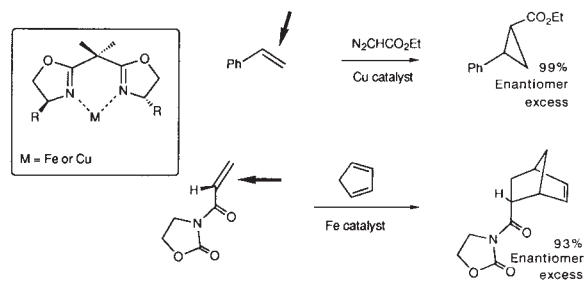


FIG. 3 Semicorrin complexes for catalytic asymmetric synthesis which enable face-specific addition to double bonds.

this paper demonstrates one of the better recent examples of how to achieve it.

In both of these catalytic reactions the excess of the desired enantiomer is high — over 99 per cent for some cyclopropanations and 93 per cent for the Diels-Alder reaction. These values are far removed from what was the norm a few years ago. The classic texts on asymmetric synthesis^{6,7} tell us that enantiomeric excesses of 20–40 per cent in stoichiometric reactions were commonly acceptable, whereas we now have the prospect of complete optical purity (100 per cent enantiomeric excess), obtained through efficient catalytic reactions, within sight. □

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Prey for the plundering

Peter D. Moore

THE study of plants that eat animals is a venerable one — Darwin devoted a treatise¹ to the subject while his German contemporary von Sachs conducted experiments² in which he was able to show that Venus' fly trap grew more vigorously when fed on a diet of woodlice than when starved of prey, thus demonstrating the nutritional value of the trapping process. But new observations and experiments, reported in *Oikos* by Zamora³, demonstrate that the plants themselves are at risk of having their hard-won prey removed by kleptoparasitic ants. Entrapped prey may seem like easy pickings for opportunist feeders such as ants, but robbing an insectivorous plant can be a risky way of gaining a meal.

Insectivorous plants have adopted a variety of techniques for obtaining prey, including passive mechanisms, such as the pit-fall traps used by pitcher plants (*Sarracenia*, for example) and the sticky traps of sundews (*Drosera*) and butterworts (*Pinguicula*), the 'steel' traps of Venus' flytrap (*Dionaea muscipula*) and the suction mechanisms of the aquatic bladderworts (*Utricularia*). Most insectivorous plants are confined to wet and nutrient-poor habitats and many are brightly coloured and conspicuous to their insect prey. Often their leaves are rich in red pigments, sometimes supplied with guide lines or other markings to lead the prey to the traps, and they may even be baited with extra-floral food, supplied as a further inducement and lure. Of the various types of trap, the pitfall and steel types are reasonably well protected against marauding scavengers, but the sticky traps are vulnerable to being plundered.

The loss of prey from the sticky-trap sundew species has been documented in *Oecologia* by Thum⁴, who found that several mechanisms could account for trap failure, including the impact of raindrops, but that theft was mainly carried out by ants. Zamora has concentrated his studies on the Spanish endemic butterwort *Pinguicula nevadensis*, found in moist locations beside streams in the Sierra Nevada of southern Spain, and has recorded systematically the prey species of this sticky trap species and those prey items preferred by thieving ants. He found that ants mainly stole larger prey and that over 50 per cent of thefts were of Diptera (flies); other important groups stolen included the carcasses of aphids and other ants. The preference was distinctly for larger prey individuals and those positioned near the edge of leaves, because these were easier to remove without endangering the individual thieves. The ants' success at avoiding capture themselves is reflected in their low representation in the normal diet of the butterwort — only two per cent of the prey captured by the plant consisted of ants. Experimental placing of ants on the leaves showed that they had a re-

markable ability to free themselves from the mucilage cover and it may well be that only the weak or the sick actually fall prey to the plant.

Zamora went further, to try to discover whether the ants' kleptoparasitic behaviour represents a serious loss of nutrition to the predatory plant. He fed 100 flies to selected plants in sites with and without high ant populations and observed the theft rate. Between 46 and 48 per cent of flies were stolen within 24 hours where ants were abundant, whereas losses in relatively ant-free areas were only 11 per cent, showing that theft could indeed be sufficient to reduce *Pinguicula* performance.

Among the sticky-trap plants, both sundews and butterworts have leaves which respond to prey capture by an incurving of leaves from the edges. This was explained by Darwin as a mechanism to enhance contact with digestive glands and to facilitate prey retention and digestion. But Zamora proposes an additional function, namely the protection of the prey items from theft. His observations certainly showed that leaf cur-

ling made it more difficult for ants to rob the plant and thefts were concentrated in the first day after capture when leaf rolling was still poorly developed. This may also account for why only large prey items could be stolen.

One speculation touched upon both by Thum and by Zamora is that the habitats of insectivorous plants may themselves be determined by ant kleptoparasitism. Are these plants effectively driven into wet (and relatively ant-free) sites by the intensity of prey theft in drier localities? There may be some truth in this proposal, but the fact remains that maintaining an exposed mucilaginous surface, as required by an efficient sticky-trap plant, demands a good water supply, and these plants are undoubtedly sensitive to drought for purely physiological reasons. Be that as it may, it is refreshing to find that so much ecological information can still be garnered by careful field observation and relatively simple experimentation. □

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HIGH-TEMPERATURE SUPERCONDUCTIVITY

Attractive vortices

P. H. Kes

RESEARCH into high-temperature superconductivity continues to generate surprises. The latest, discovered by Gammel, Bishop and collaborators¹, is that the lines of magnetic flux that thread the superconductors, when subjected to a magnetic field, can align themselves into regularly spaced chains. Peculiarly, the search for this effect was inspired by the theoretical work of Buzdin² and Kogan³ and their co-workers, but it turns out that the experimental verification is even richer than the predictions.

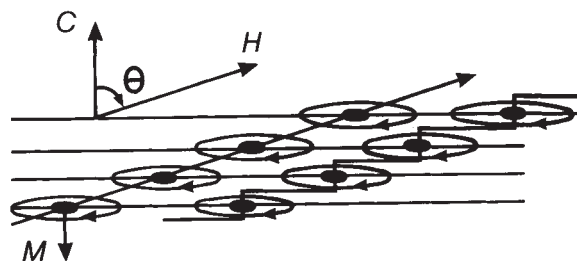


FIG. 1 Schematic view (not to scale) of two representations of flux lines intersecting CuO_2 double layers (horizontal lines) when the field H makes an angle θ with the c axis. The ellipses (lying in the $a-b$ plane) depict superconducting screening currents. To the left is the continuous flux-line picture for very anisotropic superconductors; to the right, the segmented flux-line picture with pancake vortices valid for 'Josephson' coupled superconducting layers. Note that the magnetization is directed along the c axis.

The effect is a manifestation of the layered structure of the superconducting cuprates. These materials comprise superconducting CuO_2 double (or triple) layers which are widely separated by non-or weakly-conducting metal oxide layers. This pattern leads to extremely anisotropic properties. In the compound $\text{Bi}_2\text{Sr}_2\text{CaCu}_2\text{O}_8$ (Bi:2212), for instance, the conductivity just above the critical temperature T_c is metallic in the layers ($a-b$ -planes) and is more than 10^5 times larger than it is in the perpendicular (c) direction which is semiconducting. The superconducting properties are equally anisotropic, because the CuO_2 planes carry the supercurrents while the coupling between these planes arises from the tunnelling of the Cooper pairs of superconducting electrons.

It is therefore to be expected that the anisotropy also governs the characteristics of the so-called mixed state which occurs when the material is immersed in a magnetic field, sufficiently strong ($H > H_{c1}$) for it to penetrate partially. The field penetrates along lines containing a quantized unit of flux ϕ_0 encircled by supercurrents;

these are known as Abrikosov vortices. In conventional isotropic materials, these vortices repel one another, so that the ground state of lowest energy for a flux density B consists of a two-dimensional, triangular lattice of flux lines with sixfold symmetry and lattice parameter $a_0 = (4/3)^{1/4}(\phi_0/B)^{1/2}$. The lines are aligned parallel to the applied field H and the currents circulate in planes perpendicular to H , leading to a diamagnetic magnetization M opposed to H .

In the layered cuprates, the Abrikosov lattice looks quite different, for the supercurrents are almost entirely confined to the CuO_2 planes even if the field is not perpendicular to the planes (see Fig. 1). The magnetization is thus directed along c , which leads to a torque on the material as the two fields try to align. This provides an elegant means for determining the material's anisotropy⁴. In Bi:2212 the coupling between the superconducting planes is so weak that a flux line breaks up into 'pancake vortices' as schematically displayed by the ellipses in Fig. 1. When more of these segmented flux lines are present, the pancakes in the same planes repel each other as usual.

But the diamagnetic interaction between pancakes in adjacent layers may lead, under certain conditions, to an attraction between them, so that instead of forming an elongated triangular lattice (Fig. 2a), the vortices line up in planes parallel to c and H (Fig. 2b). The flux lines in one plane form a row of tilted vortices (vortex chain). According to numerical computations (L. J. Campbell and L. Daemen, personal communication), the ground state expected consists of a lattice with distance d (fixed by the attraction) within the vortex planes and a distance c between these planes as required by the condition $B = \phi_0/cd$. It should be mentioned, in addition, that the theories^{2,3,5} ignore the segmentation of the flux lines. It is argued that the distance between the layers (at around 1.5 nm) is so small compared with either the London penetration depth (about 140 nm; the scale length for magnetic interactions) or the vortex separation (480 nm at 10 milli-

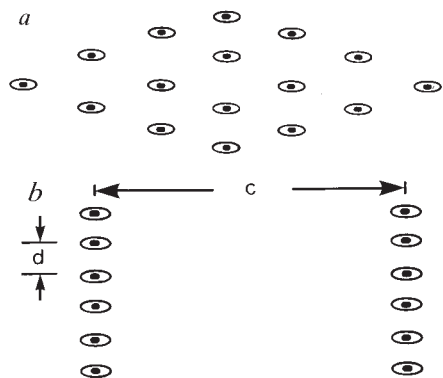


FIG. 2 Vortex positions in the plane perpendicular to H for $\theta \approx 70^\circ$. Ignoring the attractive interaction the lattice would consist of elongated triangles (a). Because the current pancakes in adjacent CuO_2 layers attract each other the vortices line up in rows (vortex chains) (b).

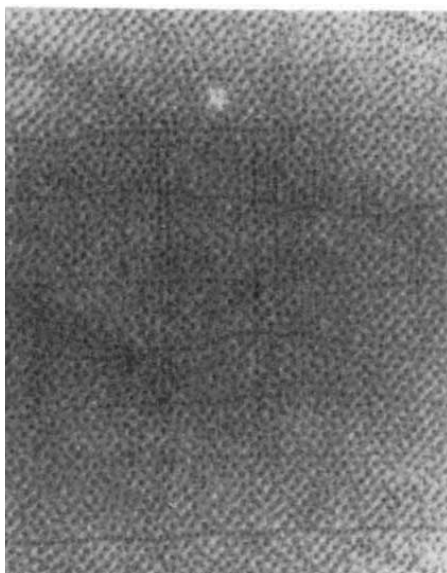


FIG. 3 Result of decoration experiment on Bi:2212 single crystal in field of 3.5 millitesla at an angle $\theta = 70^\circ$. The dark spots are vortices. Their spacing is 1.4 μm . The clearly visible chains of higher vortex density run along the direction of the field-projection on the surfaces (from ref. 1).

tesla) that the vortices can still be considered to be continuous lines (left vortex in Fig. 1) with very anisotropic interactions. Another point to keep in mind is that the formation of vortex chains is only favourable at low strength fields (much less than 1 tesla) and in a range of angles typically between 60° and 85° to the perpendicular.

In a nice series of decoration experiments¹ the Bell group tested the theoretical ideas on 100- μm -thick single crystals of Bi:2212 grown by Mitzi and Kapitulnik at Stanford University. The crystals have a thin plate-like geometry with the surface parallel to the a - b -planes. Magnetic iron particles 50 Å across are formed by evaporation in a low pressure helium atmosphere. They are preferentially trapped at the surface regions where the magnetic field is maximum, just the sites where the vortices cross the surface.

A typical Bitter pattern taken with an angle of 70° and $B = 3.5$ millitesla is depicted in Fig. 3. The average distance between the black dots indicating the flux-line positions, is 1.4 μm . Clearly observable are weakly wiggling lines with a higher vortex density. These lines are oriented along the direction of the field projection on the surface. To appreciate the pattern it should be realized that the cross-section of a vortex lattice with the surface for $0 \leq \theta < \pi/2$ will look like an Abrikosov lattice with hexagonal symmetry. It is tempting to believe that the lines of higher density display the vortex chains. The big surprise then is the presence of vortices neither by theory nor by numerical work. They predict the space between the chains to be empty.

Further decoration experiments at angles θ between 10° and 85° and in fields

from 1.7 to 9.7 millitesla show that the vortex chains occur above 60° which is in accord with theory. However, an analysis of the vortex distance in the chains shows that it scales with the average distance of the vortices between the chains. Thus it seems that the vortex separation is only determined by the field component along the c -direction. Other interesting features are the absence of long-range positional order of the flux lattice caused by the interaction with inhomogeneities in the sample (pinning centres) and the alignment of vortices along the chains. Finally, a detailed analysis shows the higher density of vortices along the chains is accomplished by interstitial vortices in such a way that there is one extra vortex along the chain for every distance c travelled.

One may conclude that there is evidence of an attractive interaction. On the other hand, the theoretical description used so far is incomplete. It is not known whether the finite thickness of the sample is important, nor is it clear whether the more exact pancake description would predict the observed patterns. One may also speculate about two classes of vortices. There is no doubt, however, that the extreme anisotropy is the underlying reason. In that sense it is worth noting that the low-field effect so nicely demonstrated by Gammel *et al.* is only the tip of the iceberg.

A flurry of recent theoretical papers⁵⁻⁹ has appeared that speculate on the properties of the vortex lattice when the field is (almost) parallel to the superconducting planes. Although these seem academic, they are of great importance for the development of conductors for superconducting magnets. The well performing tapes we hear about, with current densities of 200 A mm^{-2} in fields of 25 tesla consist of highly oriented Bi:2212 with the conducting basal plane along the tape. The field is applied along the planes but perpendicular to the current, just the situation where vortex kinks and double kinks are the most essential entities in the vortex lattice.

Kinks are a different way of representing the flux line in Fig. 1 (right) in case of extremely large anisotropy. Instead of representing it by the straight line along the field direction it makes more sense to take the zeros of the current distribution of the vortex as its definition. This line of zeros is not straight but zigzags, running parallel to the CuO_2 planes and crossing them in the centre

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of the pancakes. It is believed that the pancakes (kinks) mainly determine properties like flux pinning, flux flow and creep. The flux flow resistance, for instance, scales with $B\cos\theta$ when θ is varied from 0° to about 87° . Only in a narrow range below 90° are deviations observed¹⁰.

Because the density of pancakes scales as $1/\cos\theta$, it seems as if it is possible to ignore the real structure and to consider the pancakes to be stacked on top of each other in the c direction. This also suggests that the positions of the pancakes in each layer are independent of the positions in the adjacent layers. It is as if the layers are totally decoupled and all flux properties are determined for one superconducting layer of thickness equal to the layer spacing

(1.5 nm). This would account for the fact that large current densities in magnetic fields are only observed at low temperatures where flux creep effects are small.

Referring to the experience with the decoration experiments one may conclude by saying that theoretical models are useful and often essential, but that direct observations are always decisive. Because the Bitter technique is limited to very low fields one may hope that in the future scanning tunnelling microscopes can be developed to such an extent that vortices can be studied *in situ* at fields of several tesla¹¹. □

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ORNITHOLOGY

The question of polarization

Graham R. Martin

THERE have been many investigations of the ways that birds¹ and insects² can use the Sun's position as a means of orientation for navigation. To function as an orientation cue the Sun does not however need to be perceived directly, because sky-light contains a pattern of polarization which is related to the Sun's position³. That some invertebrates⁴ and birds can detect and employ these patterns seemed to have been well established. Now, however, experiments conducted by Coemans and colleagues of the University of Utrecht, reported in *Naturwissenschaften*⁵, have thrown work on polarization perception in birds into doubt. It may be that the phenomenon will in fact turn out to have been the product of a rather overzealous acceptance of the idea that apparently outstanding behaviour needs a 'super sensory' explanation.

The principal weakness of studies of polarization sensitivity in birds has been the failure of investigators to find a satisfactory way in which light with different planes of polarization could be discriminated. Such mechanisms have been described in certain fish retinæ⁶ and in various invertebrate eyes^{7,8} but not yet in birds. It was in looking for such a mechanism that the Utrecht team found that they could not even show that birds were polarization-sensitive.

The main problem in handling polarized light is that it is easy to translate a differentially polarized stimulus into a source of differential brightness. Light from many sources, such as a slide projector, or light that has passed through interference filters, is partially polarized. So simply putting light from such sources through a polarizing filter can induce subtle brightness patterns

which change with the orientation of the filter. If a rotating polarizing filter is used, as was the case in one experimental demonstration of polarization sensitivity in the pigeon *Columba livia*⁹, then the light pattern can become one that flickers.



The pigeon — an eye for polarized light?

Another difficulty is that many common surfaces reflect light polarized in different planes unequally. Thus an apparently uniform surface, including a matt black one, uniformly illuminated with polarized light, can become one containing subtle differences in brightness.

Coemans *et al.* believe that in earlier studies with pigeons, both sources of contamination could have influenced the results. They attempted to overcome these possible artefacts while at the same time reproducing the exact procedures that had previously given positive results. Thus, to eliminate the problem of converting a partially polarized light source into one of differential brightness, the stimulus lights were completely depolarized before being repolarized by filters.

Delius *et al.*¹⁰ had demonstrated polarization sensitivity in pigeons using a training procedure where pigeons were taught to

make key-peck responses depending upon the plane of polarization of light presented above them. In this study the walls of the response chamber were painted matt black. In the replication of the experiment, Coemans *et al.* used white blotting paper which was changed after each session. This simple difference in procedure has important consequences. In the original study the matt black surface could have produced differential reflection of the polarized light. Furthermore, such a surface may have become more reflective owing to polishing by the birds' feathers if it had not been renewed regularly. Simply changing the white paper lining of the chamber each day greatly reduced the chances that birds could learn to respond to a differential brightness cue caused by reflections within the chamber.

Using these procedures the Utrecht team were unable to find any evidence of polarization sensitivity in pigeons, although the birds did show rapid learning of other simple visual discriminations in the same apparatus. Coemans *et al.* were also unable to find any evidence that the electroretinogram of the pigeon eye showed any differential response to polarized light as had previously been reported^{9,10}, even though they used stimuli with spectral properties identical to those of the original studies.

The new investigations with pigeons also bring into question the results of field experiments with other birds, such as blackcaps (*Sylvia atricapilla*) and white-throated sparrows (*Zonotrichia albicollis*), which suggest that the polarization pattern of sky-light influences their orientation behaviour^{11–13}. These studies have involved making birds, which are in a state of migratory readiness, view a large section of the sky through sheets of polarizing filter. There is no doubt that the orientation behaviour of some, but not all¹⁴, birds is influenced by the orientation of such filters. But in the light of the findings from Utrecht it would be prudent not to assume that it is influenced simply by the plane of polarization. □

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Fresh factors to consider

Solomon H. Snyder

GROWTH factors of various chemical constitution are ubiquitous features of both plants and animals. So, some 40 years ago, it was assumed that the discovery of nerve growth factor would presage the identification of dozens of neuronal trophic proteins, yet until recently it remained the only growth factor known to have selective neuronal actions. In the past few years two proteins, brain-derived neurotrophic factor and neurotrophin-3, have been added to the family. Hyman *et al.* writing on page 230 of this issue¹, and Knusel *et al.*², now report that the first of these proteins selectively enhances the survival of dopamine-containing neurons. The finding has substantial implications both for developmental neurobiology and for the therapy of Parkinson's disease.

The identification and isolation of brain-derived neurotrophic factor (BDNF) was the culmination of an elegant and painstaking purification effort by Yves-Alain Barde,

SITES OF ACTION FOR NEUROTROPHIC PROTEINS

Neural cell target	NGF	BDNF	NT-3
Neural crest sensory	Yes	Yes	Yes
Nodose ganglion sensory	No	Yes	Yes
Retinal ganglion	No	Yes	?
Septal cholinergic	Yes	Yes	No
Substantia nigra dopamine	No	Yes	No
Forebrain GABA	No	Yes	No

Hans Thoenen and colleagues³. The cloning of a complementary DNA for BDNF⁴ was followed shortly by the identification and cloning of a cDNA for neurotrophin-3 (NT-3) in three independent laboratories⁵⁻⁷. These three proteins have about 50 per cent amino-acid sequence identity and are therefore members of the same family. But how similar are their physiological actions? Nerve growth factor (NGF) was discovered because it stimulated the growth of sympathetic neurons and sensory neurons derived from the neural crest. Almost all sympathetic nerves are stimulated by NGF, but BDNF influences only some of them. On the other hand, certain sensory neurons, such as those of the nodose ganglion, respond to BDNF and NT-3 but not to NGF (see table). One of the most exciting findings in NGF biology was that, besides its peripheral actions, NGF selectively enhances the growth of cholinergic neurons which project to the forebrain and which degenerate in Alzheimer's disease.

Neuron depletion

In the same way as Alzheimer's disease involves the progressive loss of cholinergic neurons, among others, Parkinson's disease is associated with depletion of dopamine neurons in the substantia nigra. To ascertain whether BDNF might influence these neurons, Hyman *et al.*¹ and Knusel *et al.*² applied

it to dissociated cells from the ventral midbrain of the fetal rat. Biochemical markers showed that dopamine neurons became two to five times more abundant. The effect was selective in that NGF^{1,2} and NT-3 (ref. 2) failed to affect dopamine cells.

Clinical relevance

Seeking possible clinical relevance of this finding, Hyman *et al.*¹ explored the MPTP model of Parkinson's disease. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) causes destruction of dopamine neurons and Parkinson's disease in humans. The selective destruction of dopamine neurons occurs after MPTP has been metabolized to MPP⁺ (1-methyl-4-phenylpyridinium) which is accumulated exclusively by dopamine neurons to a concentration several thousand times greater than that of the extracellular environment⁸. The application of MPP⁺ to the midbrain cells in culture led to a loss of more than 75 per cent of the dopamine neurons, whereas treatment with BDNF substantially protected against this loss. Neither NGF nor basic fibroblast growth factor, a more general growth factor with neurotrophic activities, could reproduce the effects of BDNF. Whether BDNF might prevent the loss of dopamine neurons in Parkinson's disease is unclear, as the apparent preservation of neurons may reflect only a stimulation by BDNF of those cells that survive MPP⁺ treatment. Also, the degree to which the effects of BDNF in fetal neurons can be extrapolated to adults is unknown.

Besides stimulating dopamine-containing cells, Knusel *et al.*² showed that BDNF could increase the number of GABA (γ -aminobutyric acid)-containing and cholinergic neurons as well as the amount of total neural protein. By contrast, NT-3 failed to affect cholinergic, dopamine- or GABA-containing neurons, so the main targets of NT-3 in the brain remain unknown.

In recent clinical investigations, the monoamine oxidase inhibitor deprenyl has been shown to slow markedly the progression of Parkinson's disease, presumably by retarding the loss of dopamine neurons⁹. It is possible that the controlled release of BDNF

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Ship in the night

ACTIVE galaxies are known to show all kinds of variability, but the appearance of a wholly new feature in the spectrum of one such galaxy, Markarian 231, is more of a surprise, especially as the new feature is at about one-third the redshift of the galaxy itself. This means the changed spectrum, noticed by T. Boroson *et al.* (*Astrophys. J. Lett.* **370**, 19; 1991) in a comparison between recent observations and data from 1977, cannot be due to changes in Markarian 231 itself. Instead, suggest the authors, a cloud of obscuring gas happens to have moved between us and the active galaxy sometime between 1977 and now. Absorption features due to intervening clouds are a common occurrence in the spectra of quasars and active galaxies, but little is known about the nature of the clouds. Continued observations of Markarian 231 should allow the size, density and speed of this one cloud to be measured with some accuracy, say Boroson *et al.*

src surprise

Work reported by P. Soriano and colleagues in *Cell* (**64**, 693-702; 1991) shows that *c-src* may not be as indispensable to the control of the cell cycle as its prominent role as a cellular proto-oncogene might suggest. Mice lacking functional *c-src* alleles survive for at least a few weeks after birth, implying that the gene's function may be partly complemented by other members of the tyrosine kinase family. Equally surprising is the observation that the *src*-deficient mice display a recessive form of osteopetrosis, a condition which in humans causes death at various ages due to bone malformation affecting vital organs — the gene is normally expressed at high levels in the central nervous system and blood platelets, but no abnormalities in these cell lineages were detectable in the mice.

In the swim

ONE of the most curious of fossil finds to come out of China in recent years — the diapsid reptile *Hupehsuchus* from the Middle Triassic — has been the subject of extensive preparation and thorough anatomical redescription by L. Carroll and Z. Dong (*Phil. Trans. R. Soc. B* **331**, 131-153; 1991), yet its phylogenetic relationships remain an enigma. Fish-shaped, with a laterally compressed body and flipper-like limbs, the 1-2-m-long animal was clearly adapted to an aquatic lifestyle; but the incongruously small head, with long, delicate and completely toothless jaws, is reminiscent of nothing so much as a baleen whale. Even the existence of a primitive and slightly older relative, *Nanchangosaurus*, is no help in unravelling the fossil's affinities. As Carroll and Dong point out, secondary adaptations to life in water typically make it exceedingly difficult to establish the phylogenetic position of such creatures.

directly into the substantia nigra of patients with this disease might similarly halt their deterioration. Coupled with other novel therapies, such as treatment with deprenyl, the use of BDNF could be the first of a series of advances in the treatment of Parkinson's disease, in which little progress has been made since the introduction of L-dopa (L-dihydroxyphenylalanine) in the late 1960s. Indeed, Turski *et al.*¹⁰ showed that antagonists of the excitatory amino acid glutamate, which block the NMDA (N-methyl-D-aspartate) subtype of receptor, protect dopamine neurons in the substantia nigra against destruction by MPP⁺. How might these antagonists act? The toxicity of MPP⁺ for mitochondria in dopamine neurons can lead to an increase in the permeability of ion channels

associated with NMDA receptors, an effect blocked by NMDA antagonists. Conceivably, protection of dopamine neurons may be best provided by therapy with NMDA antagonists, currently being clinically tested for the treatment of stroke, along with deprenyl and BDNF. But regardless of their possible therapeutic applications, the newly discovered neurotrophic factors are already greatly extending the scope of developmental neurobiology. □

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OBITUARY

Lord Penney 1909–1991

WILLIAM Penney, the nuclear scientist, who died on 3 March aged 81, was one of the last of the great men who could truly claim to be a 'back of the envelope' scientist. He had a brilliant mind which reduced all problems to simple essentials. This meant that he could regularly produce a good answer by lucid thinking and simple estimates. Of course he accepted the need for computers and sophisticated calculations, but insisted that everything be understood in simple physical terms. In his business that was a rare gift.

Penney did not invent the concept of the atom bomb, but he led the work for the design, manufacture and testing of the British atom bomb during the late 1940s and early 1950s. Subsequently he designed the warhead for the British hydrogen bomb, and that design was based substantially on the superb physical intuition which Penney possessed. I have heard it said that when the first British hydrogen bomb was exploded, at Christmas Island in the Pacific Ocean in 1957, only Penney was really confident that the device would work. And the computers produced the complicated implosion calculations only after the device was proven.

At an early date Penney saw that nuclear weapons had such awesome destructive power that world wars had become impossible. He worried about nuclear proliferation and worked hard with Harold Macmillan, then the British prime minister, to abolish atmospheric tests. Characteristically, his later work on the detection of underground tests led him to develop the theory of pressure pulses propagating around the Earth's surface. But despite his great scientific abilities, his common sense and integrity enabled him to make a friend of almost everyone. At one time he was chairman of the Atomic Energy Authority and his minister was Frank Cousins,

the well-known trade unionist and anti-nuclear campaigner, with responsibilities, amongst other things, for the development of nuclear weapons. In effect, the minister for nuclear weapons was against them. Nevertheless, Cousins and Penney got on well together and both carried out the collective wishes of the cabinet. That speaks well of both men.

Eventually, in 1967, Penney returned to academic life as rector of Imperial College in London, where he was a great success. Perhaps the only sadness in his professional life was his questioning by the Australian Royal Commission investigating Britain's earlier nuclear test programme on the Monte Bello Islands and at Maralinga. The scientific content of those tests was Penney's responsibility. The administrative arrangements for the tests were the responsibility of the Australian army and civil authorities. Those arrangements, like some of the early tests in the American desert, were not carried out with the same meticulous concern about radiological protection which would be considered essential nowadays. I believe Penney would have given careful and conscientious advice on all these matters, and the fault, if fault there were, over breaches of safety standards lay with the implementation of that advice.

In retirement his brain remained as active and as clear as ever. He was a director of Standard Telephones and Cables and recognized the importance of glass fibre communications at a time when most of us thought that glass fibres were for thermal insulation. Bill Penney was a great scientist and a great man. Those of us who were privileged to have known him have lost a good friend.

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Walking on water

SURF-riding seems an ideal way of exploiting wave energy. Simply by 'sliding downhill' on the upslope of an advancing wave, a surfboard can travel effortlessly at wave velocity: even faster, if it runs at an angle. Unfortunately, it only works with the steep waves of a beach. In the open ocean, waves slope much more gently, and several different wave-systems usually combine into a general chopiness that would defeat the most experienced surfer.

But Daedalus recalls his recent invention of an ultimately slippery and non-wettable surface: graphite-fluoride flock. The close-packed fibres of this material reject water and entrap air so efficiently that it presents a practically gaseous surface to the water. A surfboard coated with it would have so little drag that it could slide down a water slope of only a few degrees. Daedalus's novel 'Seasurfer' sea-going platform is supported on a multiplicity of such frictionless surfboards. At any moment, a fraction of them ride on wave-faces which slope in the desired direction. By angling these boards correctly while retracting the others, the craft will surf along the chosen course. As the wave conditions change beneath it, the craft retracts, extends, and positions appropriately just those boards needed to keep it surfing in the right direction. It will appear to be skating or dancing delicately among the waves on innumerable flat feet.

The Seasurfer will interrogate the sea beneath it by means of a grid of microwave sensors. Its on-board computer will continuously work out the disposition of surfboards that will keep the craft going in the chosen direction. If the dominant wave-pattern happens to be going that way as well, its task will be very easy; if not, the craft will have to surf at an angle to the wave-fronts, or even progress by sliding down the back faces of retreating waves. A propeller driven by the craft's progress through the water will generate electricity for the computer and actuators. Even in a very confused and choppy sea there will always be plenty of wave-faces to exploit; but in a dead calm the craft will merely rest on all its boards extended as passive floats.

The first Seasurfer model will be promoted as a sort of sea-going sun-deck for the lazier end of the yachting market. But its ultimate maritime ecological niche is hard to predict. It doesn't have to be relatively long and thin like a normal ship, but can be expanded to any shape or size. It can travel forwards, backwards or sideways with equal ease, can operate in extremely shallow water and can be safely beached in an emergency. Separate Seasurfer platforms can combine into one big craft, which can split off subunits for separate voyages or shore-visits, or even divide completely in case of mutiny. David Jones

Telomere loss and cancer

SIR — Hastie *et al.* speculated¹ that shortening of chromosome telomeres due to physiological attrition (ageing) may generate tumours. But if the loss or deletion of telomeric sequences due to senescence (or high tissue turnover) were the main factor in tumorigenesis, one would expect a low incidence of tumours in tissues such as those of the central nervous system or bone marrow stroma² normally unable to undergo regeneration. Determination of telomere length in such tissues would represent the only proper falsification context for the above speculation.

Tumours of the central nervous system, whose cells are unable to regenerate, are relatively frequent (overall incidence in the United States being 14,000 cases per year³). Moreover, most cerebral and spinal cord neoplasms are found in children, which argues against the significance of both ageing and cellular turnover in the loss of telomeric sequences and consequent cancer.

It is, therefore, not surprising (at least for a clinician) that the authors of a paper⁴ from the same research centre as that of Hastie *et al.*, suggest that, in mice, telomere size is unaffected by repeated somatic division (cellular turnover) and animal's lifespan (ageing). It appears that, in this case, clinical experience would predict that telomere shortening is unlikely significantly to influence carcinogenesis in men and mice.

Notwithstanding the above criticisms, Hastie *et al.* made an important observation that telomere lengths reflect fairly consistently the past mitotic dynamics of the somatic tissues. This may eventually be of practical help, particularly in the study of subpopulations of haemopoietic stem cells and progenitors. Also, telomere lengths may reflect generation time and cellular dynamics of leukaemic clonogenic cells, thus aiding in the design of therapeutic strategy for individual patients.

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HASTIE *ET AL.* REPLY — We hypothesized that telomere loss may play a part in generating the genetic instability observed in a particular cancer, colorectal carcinoma¹. Jankovic *et al.* attribute to us more far-reaching conclusions than we made from our study. Of course, if telomere length were the main and only factor in generating neoplasia (an improbable suggestion) we might expect a low incidence of tumours in tissues such as those of the central nervous system that have low cellular turnover. We agree that if

telomere reduction does indeed reflect cell turnover, this phenomenon is unlikely to play a role in paediatric tumours and those of the central nervous system. But the fact remains that after lung cancer, the most common cause of death due to cancer in the United Kingdom and the United States remains colorectal cancer; central nervous system tumours are extremely rare by comparison.

We also showed that mouse telomeres are very long even in old animals and concluded that telomere reduction is unlikely to play a role in cancer or ageing in mice⁵. If, as we predict, telomere loss amounts to just four base pairs per somatic cell division, we would not expect to see any measurable reduction in mouse telomeres over a two-year life span, even if such a reduction were occurring.

We feel Jankovic *et al.* are on weak footing when they surmise that if telomere loss does not play a role in carcinogenesis in mice, it is unlikely to play such a role in man. Also, at present we cannot rule out dramatic

telomere loss in mouse cancers.

We feel our most original and interesting conclusion was that telomere loss may reflect the number of cell divisions in a tissue's history, constituting a type of clock. We are now testing this idea by studying telomere length in a wide range of human tissues, including muscle and brain where cellular turnover is low.

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Measuring air from polar vortices

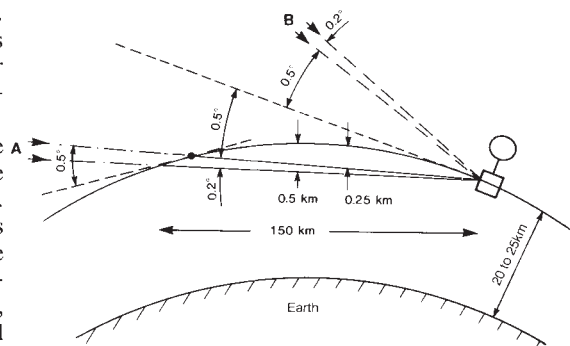
SIR—Tuck has suggested¹ that stratospheric air from within the Arctic winter vortex may be transported into mid-latitudes in cells as small as 200 km horizontally and 3 km vertically. Because such cells contain large amounts of reactive chlorine², they may be important in the ozone depletion in the Northern Hemisphere winter identified by the report of the International Ozone Trends Panel³. Cells of ex-vortex air are also characterized by a reduction in N₂O (ref. 1) and probably in other gases whose source is also in the lower atmosphere (methane, chlorofluorocarbons).

Unfortunately, such cells are barely observable by remote sensors on satellites or balloons. Because limb-sounding sensors observe horizontally through the curved atmosphere, their horizontal resolution is 400 km, which is too large. Their potential vertical resolution is 2–3 km, but, except in the case of a balloon-borne sensor, in practice this is degraded by the field of view to 5–6 km, again too large. Many constituents decrease in concentration below 20 or 25 km, which further degrades the vertical resolution. Conversely, the resolution of measurements of the source gases is improved because their concentration increases below 25 km.

Because limb-sounders are usually specified for maximum sensitivity, they observe the strongest absorption or emission lines. In the infrared, these frequently saturate at the

line centres below 25 km, broadening the atmospheric response function and further degrading the vertical resolution. Below 25 km, the sensitivity and resolution of mid-infrared emission sensors is further reduced because of the low temperature.

In designing limb-sounders to observe these cells, both horizontal and vertical resolution must be improved. The horizontal width of the field of view at the tangent point



A pseudo-*in situ* balloon-borne limb sounder for high-resolution measurements of ex-vortex air would subtract signals emitted from weak infrared or microwave lines at small angles equally above and below the horizontal (A–B). Provided the atmosphere were horizontally uniform for distances of 150 km, the dashed and dot-dashed beams would contribute equal signals; the difference signal would originate within the 0.5 km vertically below the balloon and within 150 km horizontally.

should be less than 200 km; to achieve a vertical resolution better than 3.5 km, the vertical field of view at the tangent point should be less than 1 km. At the expense of signal-to-noise ratio, the vertical resolution could be further improved by oversampling in the vertical plane and subtracting signals in ad-

jacent views. Similarly, the horizontal resolution along the line of sight of a satellite sensor can be improved by observing along the satellite track, and oversampling. Some of these improvements are included in the satellite radiometer HIRDLS (ref. 4).

Vertical resolution could also be improved by adding filters which select radiation from parts of the spectrum with weak lines, by choosing longer wavelengths where possible, and by measuring source gases. As the spectral bands of N_2O and CH_4 interfere in the mid-infrared, a radiometer that could measure a linear combination of N_2O and CH_4 would be simpler than one that measured either constituent separately, but would still be suitable for identifying exvortex air. A radiometer observing a chlorofluorocarbon at 11–12 μm would have the advantages of longer wavelength and weaker lines, but with less signal.

A balloon-borne emission sensor could achieve much better resolution by using the viewing geometry shown in the figure, not possible from satellites. By taking the difference between signals 0.6° below and 0.6° above the horizontal, with a field of view of less than 0.2° , the resolution would be better than 0.5 by 150 km. The required pointing accuracy of 0.05° is easily achieved by modern angle encoders and gyroscopes. At the expense of signal, the resolution could be improved further by reducing the elevation and depression of the viewing angles, provided that the field of view and pointing error were reduced accordingly. The measurement assumes that the atmosphere is horizontally uniform for 150 km — provided the signal-to-noise ratio were sufficiently high, this assumption could be tested by allowing the instrument to rotate freely and by comparing observations in each quadrant.

Modern limb-sounders have programmable viewing, so that the geometry of the figure could be used during ascent and a valved descent, while retaining the conventional limb scan and floating at constant altitude. Not only would this enhance the capability of general balloon-borne remote sensing, but the geometry of the figure could also be used by high-altitude aircraft provided the viewing system were interfaced to the aircraft's inertial platform.

Unfortunately, there seem to be no plans to implement the simple balloon-borne geometry of the figure, which could make observations at improved resolution and at low cost.

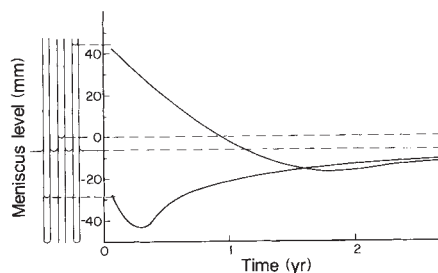
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No capillary rise?

SIR — The rise of liquid menisci in capillary tubes is always assumed to be described by the Kelvin equation¹. But I have found that the menisci in test-tubes a few millimetres in diameter, immersed halfway into a sealed reservoir of water with the open end at the top, do not rise to the Kelvin level even after several years. Whereas the menisci in neighbouring open tubes do display the expected capillary rise, those for the half-closed tubes attain the same level as that of the reservoir, irrespective of tube diameter, initial level or temperature (for $30^\circ C < T < 55^\circ C$). This behaviour is as though the surface tension of the water were zero, even though the menisci



Meniscus levels in the half-closed 4-mm diameter tubes at room temperature.

in the half-closed tubes were as hemispherical as those in the open tubes.

Six 9-cm-long pyrex tubes, three of 4-mm internal diameter and three of 1-mm diameter, were mounted, in parallel, using pyrex spacers, inside a pyrex vessel 5 cm in diameter. One tube of each size was open at both ends, and the others were closed at one end. The system was cleaned with hydrofluoric acid, detergent solution, alcohol and distilled water. The vessel was half filled with triply distilled water, pumped to the freezing point and sealed off. By manipulating the orientation it was possible to fill one of the larger tubes and have the other nearly empty, leaving both smaller tubes approximately half full. This initial manipulation also had the effect of coating all walls in the system with the original sample of water. The vessel was placed in a room-temperature enclosure and the levels were monitored with an accuracy of ± 0.01 mm.

After two years, the levels in all four closed tubes came to lie about 5 mm below the bulk reservoir level. The figure shows the results for the two 4-mm tubes. The vessel was then mounted in a thermostat set to $30^\circ C$. The levels changed at once, those in the 1-mm tubes rising four times faster than those in the 4-mm tubes; but all four levels rose only to the reservoir level.

Over the next 18 months, the temperature was raised in $8^\circ C$ steps, held at each step for several months to a maximum of $55^\circ C$, and then stepped back again to $30^\circ C$, at which temperature it was held for a final eight months. The levels in the four closed tubes did not move by more than 0.05 mm, remaining at the bulk meniscus level.

At the end of the experiment, mass spectrography showed only modest contamination of the water: boron and sodium were present at a few parts per million, as expected after the long exposure to pyrex². I found no systematic variation of impurity levels in samples from the reservoir or from the two sizes of half-closed tube. The following facts also argue against contamination effects; the internal consistency of the response to temperature changes (all four levels changed in the same way), the full hemispherical form of the menisci and the full capillary rise displayed in the open tubes, and the independence of the final meniscus levels on the initial amount of water in the tubes. The last point was checked by observing initially full and nearly empty tubes of the same radius. When these had come to the same final level, the former would have concentrated its original contaminants threefold, whereas the latter would have diluted them by half. Thus a difference of a factor of six in contaminant concentrations made no observable difference in meniscus level. This is the strongest argument against such subtle effects as the migration of minute amounts of hydrophobic impurities to the menisci. Volume-distributed contamination would anyway be expected to raise the levels in half-closed tubes above the Kelvin level, rather than the reverse, because of osmotic pressure. This is also the expected effect of fluctuations of the ambient temperature acting through the nonlinear response of the vapour pressure.

Classically, the meniscus approaches the equilibrium level exponentially, driven by an evaporation–condensation imbalance with a relaxation time of the order of one month. However, this depends on the near equality of two very large effects which may be modified differently by the presence of nearby walls. Recently, direct observation of a metastable dry state on walls only a few millimetres above a normal meniscus has been reported³. I suggest that metastable drying on the walls might be responsible for depression of the levels in the half-closed tubes in this experiment. (These experiments were performed at the University of Toronto.)

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of fewer than 500 words and five references. □

Pushing the pill

Walter Gratzer

Génération Pilule. By Étienne-Émile Baulieu. Editions Odile Jacob (Paris): 1990. Pp.314. FF130.

PHILLIP Larkin put it like this:

*Sexual intercourse began
In nineteen sixty-three
(Which was rather late for me) —
Between the end of the Chatterley ban
And the Beatles' first LP*

But in truth liberation came with the Pill, which, in Étienne Baulieu's words, dissociated the act of love from procreation and became at once part of the currency of dinner-table conversation, just like condoms 20 years later. It was rumoured that the august Catholic weekly, *The Tablet*, uneasy at the malign interpretation that might be placed on its name, would soon change its masthead and wags suggested that a fitting alternative would be *The Safe Periodical*.

Étienne Baulieu had already made his mark in endocrinology when the pill came to fruition, and in fact its true begetter, Gregory Pincus, had taken a kindly interest in the young man's career and seen to it that he received recognition and support for his research. France, to be sure, did not afford the ideal climate for work in this area, for the Law of 1920, which forbade the use of contraception, remained in force until 1966. Baulieu played his part in bringing about the repeal of this preposterous anachronism as one of a committee of 13 'sages', set up by the Minister of Health to report on the matter. Yet the fact remained, as Baulieu records, that neither academic science nor the pharmaceutical industry were willing to court social and political opprobrium by engaging in research on contraception; and so Roussel-Uclaf, a company long noted for its excellence in steroid research, hung back and left the market to Schering and Organon.

As a consultant to Roussel, Baulieu took much of the credit for changing the management's posture. INSERM had set him up with a research unit in a medical school and his work there on the progesterone receptor as a target for biochemical intervention in the development of pregnancy led eventually to the discovery of the now celebrated progesterone analogue, RU 486 — short for Roussel-Uclaf's compound 38486; their chemists, as Baulieu observes, had not been idle. RU 486 bound with high avidity to the uterine progesterone receptors, but unlike other analogues did not set in train the events associated with the binding of the hormone itself. This was a slice of luck, which Baulieu and his colleagues knew well how to exploit. Clinical trials followed with remarkable despatch, and eventually, when combined with the administration of a prostaglandin to provoke contractions, RU 486

gave a 95 per cent tally of success in the expulsion of early embryos. Patients and doctors, not to mention Baulieu and Indira Gandhi, were delighted.

Uproar followed. The Cardinal Archbishop of Paris declared that life began at conception, no matter, as Baulieu points out, that no priest ever prayed for the souls of the pre-embryos that are spontaneously eliminated with high frequency, often unnoticed. In a television debate Baulieu was unfavourably compared to Hitler and Stalin by a fellow-professor, the Right-to-Lifers marched in Washington, and Roussel-Uclaf and their masters, Hoechst, took fright and revoked the plans to market their compound. It took a threat from the French government (which has a share in Roussel), that they would reallocate the patent, to bring them to heel. The struggle continues, but RU 486 now gives the brightest hope for containing the population explosions in the East and saving the lives of untold numbers of women, who die each year from botched abortions.

Étienne Baulieu has written an engrossing book. His account of how RU 486 came to be discovered and marketed is complemented by a long appendix — the *Cahier Scientifique* — which summarizes the physiology and biochemistry of the reproductive cycle with exemplary clarity.

Génération Pilule is besides a personal memoir of Baulieu's life and career. His father, a professor of medicine at Strasbourg, had been drafted into the German

army in the First World War and decorated with the Iron Cross; but, like most Strasbourg bourgeois, he was absolute for France and found an ingenious way of serving his country: he asked the German officers among his patients to record for him at regular intervals after their discharge from hospital — for he was a kidney specialist — the volume of urine that they were producing. The reports came in from the front and allowed him to infer the whereabouts of the units to which his patients belonged. He was eventually found out and forced to run for it, but he lived to receive the *Croix de Guerre* as a reward for his patriotism.

Étienne Baulieu, we learn, is a *nom de guerre*, for in 1942 its young bearer joined a Communist youth group, allied to the Resistance, and took false papers. Later he was assimilated into the army; demobilized, he entered medical school, and resolved to keep the name by which his friends knew him. Moreover, he was conscious that even in the dawn of postwar renewal he stood a better chance of advancement in French professional circles as Étienne Baulieu than Émile Blum.

Baulieu has some telling anecdotes of his early career, of his friends and his patrons, who included the blind biochemist, Max-Fernand Jayle, his enemies (no negligible number, by his own account) and the Byzantine turmoils of French science. Self-effacement is not his style: his relish for a good punch-up, his appetite for competition and for publicity, his enthusiasm for science and the forthright pleasure that he takes in his successes make a striking contrast to the polite reticence of many recent Anglo-Saxon memoirs of the scientific life. □

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Early Egyptology — scientific examination of a mummy at Manchester University in 1908. From *Mummies, Myth and Magic* by Christine El Mahdy (Thames and Hudson) £8.95.

On the plate

Peter J. Smith

Global Tectonics By Philip Kearey and Frederick J. Vine. Blackwell: 1990. Pp.302. Hbk £35, \$78.95; pbk. £16.50, \$37.95.

A QUARTER of a century has now passed since the widespread acceptance of continental drift, the discovery of seafloor spreading and the development of these, and other phenomena into the theory of plate tectonics (global tectonics) wrought the most profound revolution the Earth sciences have ever seen. Yet despite the upheaval that the new view of the Earth has caused and the influence of the revised perspective on almost all aspects of the study of the solid Earth, there have been surprisingly few books devoted solely to it, as opposed to the way it has contributed to particular fields.

Over the years there have been one or two advanced research monographs, several historically-based accounts, a number of compilations of key papers, a few brief and limited 'popular' versions and at least one very short basic text (D. C. Heather's *Plate Tectonics*, Arnold, 1979) — which Kearey and Vine fail to cite; and, of course, almost all general geology texts these days are informed by plate tectonics to a greater or lesser extent. But with the exception of A. Cox and R. B. Hart's *Plate Tectonics: How it Works* (Blackwell, 1986), which is concerned primarily with the detailed mathematical and geometrical aspects of plate kinematics, I can think of no single book in 25 years that could be regarded as a basic text on global tectonics *per se*, although P. J. Wyllie's rather general *The Way the Earth Works* (Wiley, 1976) could perhaps be considered a contender.

Kearey and Vine have thus filled what reviewers, in their less inspired moments, are wont to call an 'obvious gap' in the scheme of things. Obvious though the gap may have been, however, it has hitherto been by no means clear just what such a text should contain, beyond the basic framework. So pervasive is the concept of plate tectonics now that one could build even a basic text into a monument; and Kearey and Vine's greatest problem must surely have been how to decide what to leave out in order to produce a book of, in their words, 'manageable length and reasonable price'. What, then, have they chosen to put in?

The three chapters following the brief opening 'historical perspective' are what might be called plate-tectonic scene-setting, covering, respectively, the interior structure of the Earth (seismology, seismic tomography, crustal and mantle structure and deformation, the core, isostasy, heat flow, ophiolites, the Earth's composition), continental drift (palaeomagnetism, palaeoclimatology, continental reconstruction) and seafloor spreading (geomagnetic reversals,

magnetic anomalies, magnetostratigraphy, deep-sea drilling, the Vine-Matthews hypothesis). It is already clear by this stage, therefore, that the book covers, albeit briefly, a good part of geophysics and touches on a number of other subjects besides, all of which preface a chapter on the 'framework' of plate tectonics (plates and plate boundaries, earthquake distribution, relative and absolute plate motions, the forces acting on plates).

Thereafter, four chapters deal in rather more detail with the nature of, and the processes taking place at, the various types of plate boundary — namely, oceanic ridges/continental rifts, transform faults, subduction zones and mountain ranges (both continental-continental collisional and Andean type). The main thrust of these excellent summaries is inevitably geophysical, although there are contributions from petrology, volcanology, sedimentology, metamorphic studies and more general geology (for example, of suspect terranes, which, curiously, are misspelled terrains'). The penultimate chapter then deals with the possible mechanisms of plate tectonics, whilst the last is a miscellaneous collection of topics, including continental splitting, the Wilson cycle, Palaeozoic and Precambrian plate tectonics, and plate tectonics in the context of economic geology.

Earlier, I implied that this is the much-needed basic text on global tectonics, and it is in a way — but not in every way. As far as the coverage is concerned, it fits the bill entirely, but the authors' claim to have aimed the book at 'senior undergraduate... and postgraduate students' has to be taken seriously. Thus if I have one disappointment it is that from the teaching point of view the book is not quite basic enough. Despite the inclusion of a rather half-hearted list of 50 'review questions' (for which no answers are provided), it is not a true textbook, partly because it is fully referenced as a research monograph and, more seriously, because it too often fails to explain.

In one pair of adjacent paragraphs, for example, we are introduced to the terms 'newtonian fluid', 'superadiabatic temperature gradient', 'kinematic viscosity' and 'dynamic viscosity' with no definition of any of them; two do not even appear in the rather skimpy index. It is just a pity that for the sake of a very modest increase in length, this first, fine attempt to provide a comprehensive summary of basic global tectonics could not have been made rather more accessible to a wider range of students. Useful though the book is, *Global Tectonics — The Textbook* remains to be written. □

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■ Of related interest is *Shaping the Earth: Tectonics of Continents and Oceans* edited by Eldridge Moores (Freeman; £10.95, \$11.95).

Mind-forged manacles

Frederick Warner

The Genocidal Mentality: Nazi Holocaust and Nuclear Threat. By Robert Jay Lifton and Eric Markusen. Macmillan/Basic Books: 1990. Pp. 346. £20, \$22.95.

IT IS A good time to look at our attitudes to war. The psychiatrist and sociologist authors of *The Genocidal Mentality* consider that the evidence of the Holocaust and nuclear bombs on Japan favours the conclusion that American scientists are a long way down the road to a genocidal mentality. Their 'mind-set' is established by the psychological mechanisms of 'psychic numbing' and 'doubling', dissociative processes enabling us to remain sane in the service of social madness. They were evident in the Nazi doctors and psychiatrists who progressed from the advocacy of eugenics through the elimination of genetically disabled individuals up to the final solution of destroying the Jews.

This progression is said to be mirrored in the 1956 report on the preparation of the American civilian population to accept a nuclear weapons programme and the possibility of annihilation. The report was requested by President Eisenhower from a panel including 11 white males of whom five were physicians, three of them psychiatrists. To quote: "This involvement of professionals is troubling but not surprising. For professionals tend to work from, rather than to question, social norms. By exclusively stressing their technical function, they create what William Blake called 'mind-forged manacles'." They provided the first line of acceptability. The second line has an ethical base, acceptance of the 'just war' with the principles of discrimination and proportionality to reduce risks to noncombatants. The third line of acceptability is to envisage a shield against nuclear weapons, the 'Star Wars' technology. This has recently found support in the success of the Patriot against Scud missiles fired at Israel and Saudi Arabia.

These are the mechanisms for harnessing the enthusiasm of scientists to perfect more and better weapons. Also "camaraderie, intense male bonding — especially when probing into arcane, forbidden areas — can provide a rare intimacy and ease of being". Yet there have been the successes of the Test-Ban Treaty, the SALT negotiations and later INF agreement which show a halt and reversal of growth. Proper credit in this process must be given to scientists in the United States and the Soviet Union. The US National Academy of Sciences has a special committee composed half of government scientists and half independent who discuss nuclear weapons and their use.

Popperfoto

Finding a pill for every ill

Joe Collier

In Search of a Cure: A History of Pharmaceutical Discovery. By M. Weatherall. Oxford University Press: 1990. Pp.298. £19.50, \$45.

Drug Discovery: A Casebook and Analysis. By R. A. Maxwell and S. B. Eckhardt. Humana Press: 1990. Pp.439. \$79.50 (US), \$89.50 (export).

SOCIETY has struggled to develop ways of allaying suffering. Of the various alternatives it is to medicines that most turn when troubles arise, and our dependence on them has increased as they have become more effective and less likely to cause damage. Gradually the prospects of providing a 'pill for every ill' grow closer and the chances of fulfilment improve as developers harness efficient methods for drug discovery, but what best to harness is still not clear. To some, investment in the scientific approach offers the surest route, for they see the development of drugs as a critical scientific process in which step by logical step dividends accrue. To others it is a more whimsical process reflecting the almost magical nature of drugs themselves, and for them, investing in a free intellectual atmosphere would seem more appropriate. Neither is a new stance, echoing, as they do, T. H. Huxley's description of drugs as "cunningly devised torpedoes" and Paul Ehrlich's as "magic bullets".

It is difficult to escape the magical lobby. Homeopathy and allied 'alternative' medicines are increasingly popular but their origins and mechanisms remain scientifically insoluble. The placebo, the lynch pin of scientific process, still defies explanation, and scientists hardly need reminding that when their methods are given their head they "can't even find a cure for the common cold". Nevertheless there has been a continuous shift from magic to science and the change is well illustrated by studying the history of drug development. It is the evolution of this process, and an assessment of the present state of the art, that are dealt with in these two books. *In Search of a Cure: A History of Pharmaceutical Discovery* by M. Weatherall essentially analyses the concepts and processes that have led over the years to drug treatments, the last entries being from early this century. *Drug Discovery: A Casebook and Analysis* by R. Maxwell and S. Eckhardt deals with issues since the 1940s, and reports on a survey analysing the intellectual methods used in the discovery of drugs deemed truly innovative.

Of the two books Weatherall's is the more eloquent, absorbing and to my mind, more stimulating. In a very 'western', and occasionally idiosyncratic approach, Weatherall describes key intellectual, methodological

Crime against humanity — a dead man holding a baby after an Iraqi chemical-bomb attack on a Kurdish city in northeastern Iraq.

There is a corresponding committee in the Soviet Union Academy and the two hold joint meetings from time to time. An attempt to encourage formation of a similar committee from the academies of Europe failed. What has succeeded on an international level is the ICSU-SCOPE study from 1983 to 1988 on environmental consequences of nuclear war with successive stages of targetting (1) other weapon sites (counterforce), (2) military installations (extended counterforce), (3) industrial sites, and (4) infrastructure, with increasing civilian casualties. The final effects of exchanging 50,000 nuclear warheads in the northern hemisphere would be 100 million to a billion deaths with 100 cities of over one million people set on fire. The smoke could cause a temperature drop of 10 °C or more over some months with the loss of a year's harvest if the drop was in early summer. The remaining population of North America could survive on stocks but not that in the Soviet Union where there are no stocks, nor in China, India or southeast Asia.

This study has stood up to criticism and sensitivity analysis and helped the scientific backing for reduction of arsenals, and the avoidance of a 'nuclear winter' possibility. The authors' prophecy of genocidal mentality then comes into question, and their analogy with the Nazi holocaust. The insight into psychological mechanisms which led a whole country to ignore what was happening is valuable. Those who knew were able to lead divided lives, and promote the collusion which even led victims to become victimizers. A democracy such as the United States cannot fail to note such warnings.

Inevitably there will be similar arguments

about the Gulf war, whether it is just or can be justified and whether the deaths of civilians fall within the idea of proportionality. The Archbishop of York, John Habgood, wrote in 1977 "Part of the Christian conscience over modern warfare lies not only in the devastation it can cause physically, but in the absence of the kind of personal relationships between combatants, which in other circumstances can impose their own restraints". He saw a compensating factor in the growth of mass communications so that television can show what is happening, but it is not easy. The Iraqis recently televised the deaths in what they called a civilian shelter and the allies a command-and-control centre, hardened against the electromagnetic pulse and operating up to the moment of attack. This is a testing time for C³I — the four legs of strategy — command, control, communications and intelligence, especially for the last which has to produce legitimate targets and safeguard proportionality.

The authors' plea is for species awareness to counter nuclearism and the genocidal mentality associated with it. It seems out-of-date now there are radical moves to limit weapons and an expanding interest of scientists in ecosystem protection, climate modelling and general environmental studies. Saddam Hussein has not needed scientists or nuclear weapons for genocide among Kurds or for using oil to pollute the seas and atmosphere. Some day the authors may help us to understand the psychological mechanisms behind this and be better prepared to avoid war. □

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and material discoveries that have lead to the cures that now support us. The book is presented in a standard history format as it traces the development of 'cures' since the Greeks, stressing particularly those advances that had bearing on matters pharmaceutical. Topics are covered in historical order with each chapter dealing with a different theme ('The Chemistry of Medicines', 'Replacement Therapy', and so on). As developments quickened, themes have necessarily overlapped and Weatherall gives helpful reminders with cross-referencing.

In Search of a Cure is by no means a complete account and in selecting his particular landmarks Weatherall essentially overlooks, for instance, Boyle, Descartes and Morgagni and developments in the Chinese or Indian subcontinents. But the subjects he does cover are complete in themselves, are well referenced, are described with clarity and insight and are skilfully woven into the context of their times. Friedrich Wöhler's discovery in 1828 that organic compounds synthesized in the laboratory could be structurally identical to those produced by the body was met with resistance, not least from his mentor, Jöns Jakob Berzelius, for it questioned the dogma of vitalism. But the relief felt by the more analytical of the time must have been enormous, and this sense of relief is transmitted to the reader.

But what makes this book so entertaining is that in addition to the science, time and again the reader is trusted with the author's asides. How the great Robert Koch argued that there was virtually no danger of humans catching tuberculosis for cattle, how throughout its history the pragmatic business approach rather than the academic has dominated the pharmaceutical industry, how a merchant banker was the father of epidemiology, and how, according to Weatherall, much of Alexander Fleming's fame has been the product of the media in which Fleming's ease with the press contrasted with Howard Florey's media phobia.

This is a good read. It leaves one in no doubt that the scientific process has been a dominant feature in the evolution of drug discovery but luck has played a major part. Time and again ideas moved between minds, disciplines, countries and up and down false paths till chance was given the opportunity

to "favour the prepared mind".

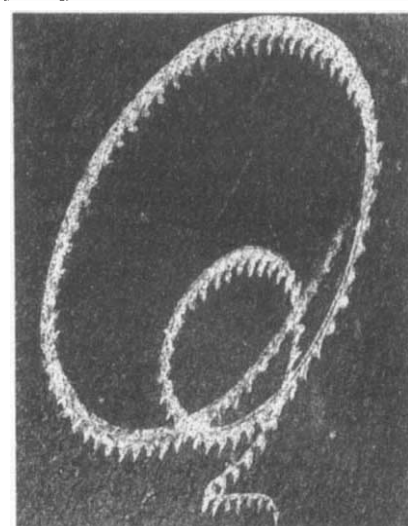
As much as Weatherall is absorbing, I found Maxwell and Eckhardt annoying. *Drug Discovery: A Casebook and Analysis* is a hotchpotch of concepts which ultimately provides little intellectual advance. "Leading North American clinician-scientists" (selection criteria unstated) were asked by an "informal written poll" (questions not stipulated) about innovative therapeutic agents introduced since the Second World War. All drugs (with the exception of those used for the chemotherapy of infection or cancer) appearing in more than 50 per cent of a minimum number of ten returns (rather arbitrary) were then submitted to an analysis designed to check whether they were indeed innovative, whether or not the discovery was the product of chance or design (assessed from analysis of the original publications and contemporary literature), where they originated (country, type of institution) and which was the primary academic discipline involved (for example, pharmacology, chemistry). Each chapter relates to a different drug (32 in all), and is presented in one of seven sections depending on its broad therapeutic category (for example, 'Rheumatology', 'Gastrointestinal', 'Neurology'). An eighth section deals with analysis and interpretation.

The bulk of the text consists of rather heavy descriptions of the drugs themselves, first 'defending' their inclusion in the study as innovative, then describing how their use had altered clinical practice. The remainder deals with the study proper, analysing the pedigree both of the drug and its inventor(s).

The findings are perhaps much as to be expected. We learn that most of the ideas crucial to recent drug development have emanated from the United States; that 60 per cent of the drugs were developed solely in one country, the remainder involving contributions from two or three; that the predominant intellectual drive behind each discovery was shared roughly equally between industry and university; that drug research never seemed to follow plans designed at the outset; and finally that there was a general and 'respected' role for a widespread contribution of serendipity.

It is tempting to accept these findings but there are aspects which undermine confidence; the essentially bogus distinction between good luck and serendipity, the belief that data taken from published material can provide a full picture of the process and the reticence of the authors to approach the inventors themselves for fear of getting unreliable or partisan information, are all worrying. In their next book, and others are promised, perhaps Maxwell and Eckhardt might start with a section validating their own survey methods. □

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GRAPTOLITES are the perfect fossils. Beautiful as objects and relatively common, they have long been prized by collectors. But totally extinct and unlike any living creatures, their biology and phylogenetic relationships have been a puzzle for professionals. Enough is known, though, to allow the production of *Graptolites*, edited by Douglas Palmer and Barrie Rickards, a concise yet lavishly illustrated guide to the group. Published by Boydell and Brewer (price £39.50, \$79), it is a pilot in what promises to be an excellent series of users' guides to all fossils.

Graptolites were marine, colonial animals that lived between about 550 and 300 million years ago. The individuals, or zooids, were linked together with common soft tissue and housed in a tough, collagenous communal exoskeleton. The distinctive shapes of the exoskeleton, as well as being visually appealing (the fossil in the picture, which is taken from the book, is *Monograptus proteus*) are essential for identification. Benthic, epibenthic and planktonic forms existed, and the animals reproduced either asexually (by budding) or sexually. Opinions vary about their affinities: some researchers align them with colonial coelenterates, but a relationship with colonial hemichordates is the majority view.

Graptolites tells you what the animals were made of, how they lived, where to collect the best ones, which museums hold the best collections, addresses of graptolite experts and concludes with 138 full-page, black-and-white plates. The chapters are short and written in a crystal-clear style that falls exactly midway between basic introduction and professional guide, with the best bits of both and the bad bits of neither. The editors have conducted this collaborative venture with considerable editorial aplomb, including nothing superfluous and yet leaving nothing out. A perfect book, then, for the perfect fossils.

Henry Gee

New in paperback

■ Of interest to students, researchers and technicians in the biological and biomedical sciences is *An Introduction to Centrifugation* by T. C. Ford and J. M. Graham. The book is published by Bios Scientific at £12.95, \$25.

■ The fourth edition of *Lipid Biochemistry: An Introduction* by M. I. Gurr and J. L. Harwood has just been published by Chapman and Hall. Price is £19.95, \$39.95.

■ *Peptide Hormone Action: A Practical Approach* edited by K. Siddle and J. C. Hutton provides a useful summary of laboratory methods and experimental ideas. Published by Oxford University Press/IRL Press at £22.50, \$50. □

A superantigen encoded in the open reading frame of the 3' long terminal repeat of mouse mammary tumour virus

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Mice express a collection of superantigens, which bind to class II major histocompatibility proteins and interact with T cells bearing particular V β chains as part of their $\alpha\beta$ receptors. These superantigens have been suggested to be encoded by exogenous or endogenous mouse mammary tumour viruses. One such superantigen is now shown to be encoded in the open reading frame of the long terminal repeat of a mammary tumour virus, a gene of previously unknown function.

OVER the past 20 years a series of alloantigens, now termed superantigens, have been identified in the mouse which combine with class II molecules of the major histocompatibility complex (MHC) to form ligands capable of interacting with a high proportion of T-cell $\alpha\beta$ receptors. This high frequency occurs because receptor recognition of superantigen-MHC complexes requires only an appropriate receptor V β element, with little contribution from the other variable components (V α , J α , D β , J β)¹⁻⁴. As alloantigens, superantigens often induce powerful responses *in vitro* from T cells bearing the appropriate V β elements⁵⁻⁷. When expressed by mice themselves, as self-antigens, superantigens cause the elimination of T cells bearing target V β s during the establishment of self-tolerance¹⁻⁴. The molecular nature of these superantigens has remained a mystery, although the genes encoding many of them have been mapped and are scattered throughout the mouse genome^{4,5,8-10}. One of these superantigens, specific for V β 14⁺ T cells, was encoded by the exogenous milk-borne mammary tumour virus (MTV) of C3H mice¹¹. Genetic mapping data indicate a strong association of many of the other endogenous mouse superantigens with various proviral MTV integrants^{4,12-16}, raising the possibility that most, if not all, of the murine superantigens are encoded by MTVs. We have now attempted to identify which gene in the milk-borne MTV encodes the viral superantigen (vSAG).

MTV 3' LTR ORF encodes superantigen

Like other retroviruses, MTVs have *gag*, *pol* and *env* genes which produce multiple polypeptides¹⁷. Unlike other murine retroviruses, however, MTVs have an open reading frame (ORF) in their 3' long terminal repeats (LTRs) capable of coding for a protein with a relative molecular mass as great as 37,000 (M_r 37K); no viral function has yet been found for this protein¹⁸⁻²³. Although the ORF gene is highly conserved among the MTVs, there is about 10-15% variation in amino-acid sequence between different ORFs, which could account for the different V β specificities of the MTV-associated superantigens. We therefore suspected that this gene might encode the V β 14-specific superantigen.

The LTR ORF of the milk-borne C3H MTV was subcloned into an expression vector as described in Fig. 1. This construct

was transfected into the B-cell lymphoma, LBB.1 (ref. 24). After drug selection the untransfected cell line and several independent transfectants were tested for their ability to stimulate the production of interleukin-2 by a V β 14⁺CD4⁺ T cell hybridoma, K14-15.11. Some of the results are shown in Fig. 2. Although untransfected LBB.1 failed to stimulate K14-15.11, three of four ORF-transfectants of this line were active. LBB.1-ORF9 was the most effective, LBB.1-ORF4 was intermediate, LBB.1-ORF8 less so, and LBB.1-ORF1 had no activity. Similar results were obtained in three additional experiments.

To confirm that the ORF gene encoded the vSAG, we tested LBB.1 and one of its ORF transfectants, LBB.1-ORF9, for their ability to stimulate normal T cells *in vitro*. We used CBA/J T cells as responders because tolerance to the superantigens in this strain deletes T cells bearing V β elements capable of responding to the known MTV-associated superantigens expressed by untransfected LBB.1 (M1s-1^a, M1s-3^a, Etc-1, and so on¹⁻⁴). Therefore, response to these antigens could not obscure the potential response of V β 14⁺ T cells to the transfected vSAG in this experiment. This point is demonstrated by the lack of T cells bearing V β s 3, 5, 6, 8.1, 11 and fewer V β 7-bearing T cells in strain CBA/J (Table 1). Despite this precaution, however, a vigorous proliferative response was seen when

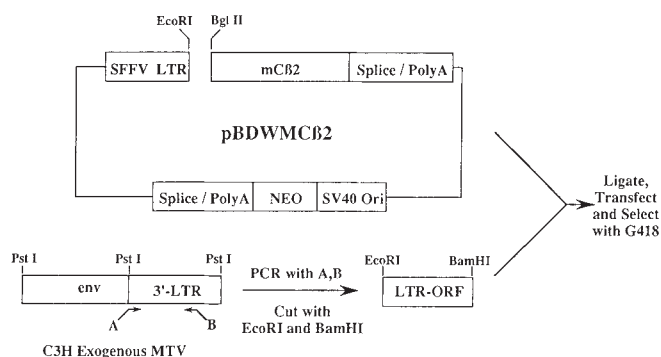


FIG. 1 Construction of a C3H exogenous MTV LTR ORF expression vector. The MTV LTR ORF coding region was amplified from a fragment of MTV containing *env* and the 3' LTR (a gift of J. Majors) by a polymerase chain reaction using primers A and B which contained *EcoRI* and *BamHI* sites, respectively. The sequences of the primers were as follows: A, 5'-GGGAATTCCTCGAGATGCCGCGCTGCAG-3'; B, 5'-GGGATCCTCTAGAGGGAACCGCAAGGTTGGG-3'. The amplified LTR ORF fragment was cloned into plasmid pTZ18R, sequenced and then ligated into the eukaryotic expression vector pBDWMCB2, as previously described⁴⁵. This vector contains a 3' β gene. The final expression vector, pBDWMCB2-ORF, was transfected into different B-cell lines by electroporation and transfectants were selected with G418⁴⁶. Plasmid pBDWMCB2-ORF is predicted to encode a 2.8-kilobase (kb) mRNA which contains the LTR ORF as a coding sequence and mouse TCR β 2 as part of its 3' untranslated region. Abbreviations: SFFV LTR, spleen focus-forming virus long terminal repeat; SV40 ori, simian virus 40 origin; env, MTV envelop; LTR, MTV long terminal repeat; LTR ORF, MTV long terminal repeat open reading frame.

TABLE 1 Response of CBA/J CD4⁺ T cells to ORF-transfected LBB.1

V β	Known super Ag in CBA/J	% of CD4 ⁺ CBA/J T cells bearing particular V β elements		
		Unstimulated	LBB.1 stimulated	LBB.1-ORF9 stimulated
3	Mls-3 ^a	0.2	0.4	0.1
5.1-2	Etc-1	0.1	0.5	0.1
6	Mls-1 ^a	0.1	0.5	0.2
8.1	Mls-1 ^a	0.0	0.0	0.8
9	Mls-1 ^a	0.2	1.7	0.7
11	Etc-1	0.1	0.7	2.1
7	Mls-1 ^a	4.5	77.7	55.3
2	None	7.9	3.0	2.6
4	None	11.3	3.9	5.7
8.2	None	14.3	5.1	5.1
8.3	None	11.2	5.1	0.0
14	None	10.1	3.9	17.5
	Total	60.0	102.5	90.2

T cells were purified from the lymph nodes of CBA/J mice and analysed for V β expression as previously described^{1,2}. CD4⁺ lymph-node T cells were isolated by passage over nylon-fibre columns and panning on anti-CD8-coated plates as previously described⁵². These cells were then cultured at 2×10^5 per ml for 3 days with mitomycin-C-treated LBB.1 or LBB.1-ORF9 cells (2×10^5 per ml) and irradiated (1,000 rad) CBA/J spleen cells (3×10^6 per ml) as 'fillers'. Recombinant human interleukin-2 (20 U ml^{-1}) was added to the cultures on day 3. On day 4, live T cells were purified and cultured for a further 2 days in interleukin-2. T cells were then stained and analysed for V β and CD4 expression¹. The V β specificities of the superantigens in CBA/J mice are taken from refs 2-4, 6 and 9.

CBA/J T cells were cultured with either LBB.1 or LBB.1-ORF9. CD4⁺ T cells in the resulting blast populations were compared for frequencies of cells bearing V β 14⁺ or any of the other V β elements against which we had an antibody. The frequencies of T cells bearing V β 3, 5, 6, 9 and 11 stayed very low and the frequencies of T cells bearing V β 2, 4, 8.2 and 8.3 decreased in both populations. As predicted, the frequency of V β 14⁺ T cells decreased in the population responding to untransfected LBB.1 and expanded considerably in the population responding to ORF-transfected LBB.1. Surprisingly, despite the partial deletion of V β 7-bearing T cells in CBA/J by MTV-7-associated Mls-1^a, residual V β 7⁺ T cells were greatly expanded in both populations. Similar results were obtained in a second experiment. These results confirmed that ORF encoded by the

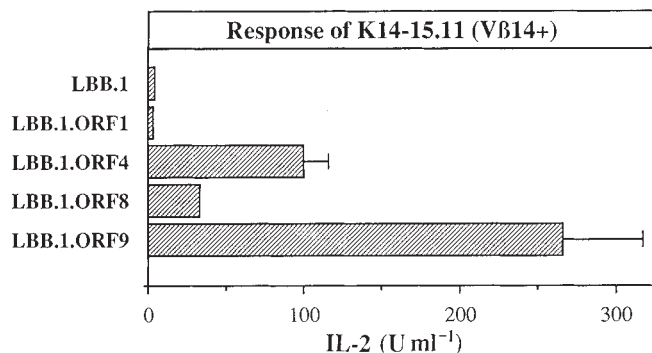


FIG. 2 MTV ORF products expressed by a B-cell transfectant stimulate a V β 14-bearing T cell. The B-cell lymphoma hybridoma, LBB.1²⁴, and derivatives transfected with MTV ORF were used to stimulate a V β 14-bearing T-cell hybridoma, K14-15.11. The response of the T-cell hybrid was assessed by lymphokine production as previously described. K14-15.11 was the product of fusion between an $\alpha^- \beta^-$ derivative of the thymoma, BW5147⁴⁷ and V β 14⁺ B10.BR T cells, produced after stimulation with the anti-V β 14 antibody, 14-2 (ref. 48; gift of D. Raulet).

C3H/HeJ exogenous virus was a V β 14-specific vSAG and indicated that LBB.1 and its ORF transfectant must express a previously unknown V β 7-specific superantigen. If this is an MTV proviral vSAG then comparison of the MTV proviruses known to be present in either RF or BALB/c mice, but absent in CBA/J mice, suggests that either MTV-1 or MTV-29 encodes this antigen.

V β 14⁺ and V β 15⁺ T cells respond to ORF product

The anti-V β monoclonal antibodies used in this experiment accounted for the V β s borne by about 60% of unstimulated CBA/J CD4⁺ T cells and all of the LBB.1-stimulated T cells, but only about 90% of the LBB.1-ORF9-stimulated T cells. This result suggested that T cells bearing a second V β element may respond to the ORF-encoded vSAG from the exogenous virus. We tested this possibility in two ways. First, we used a panel of T-cell hybridomas, including some bearing V β elements not covered by the set of antibodies used above (V β 1, 10, 13, 15). These hybrids were challenged with LBB.1 or LBB.1-ORF9. The results are shown in Fig. 3. None of the hybridomas responded to LBB.1. Both V β 15⁺ hybridomas tested responded to LBB.1-ORF9, strongly suggesting that T cells bearing this V β , as well as those bearing V β 14, respond to this vSAG.

The ability of the ORF-encoded vSAG to stimulate V β 15⁺ T cells was confirmed in a second experiment in which the LBB.1-ORF9-responsive T-cell blasts (Table 1) were used to produce T-cell hybridomas². Consistent with the staining data, 60% of these hybridomas bore V β 7, 20% V β 14 and 20% a V β element which could not be identified with anti-V β antibodies (data not shown). Representative hybridomas of each type were tested for their ability to respond to either LBB.1 or LBB.1-ORF9. As expected, all of the V β 7⁺ hybridomas responded both to LBB.1 and its ORF-transfectant (data not shown). The results with the other hybridomas are shown in Table 2. Eight of the nine V β 14⁺ T-cell hybrids responded to LBB.1-ORF9, but not to the untransfected LBB.1. The exception responded to neither. Four of the hybridomas bearing unknown V β s responded to LBB.1-ORF9, but not LBB.1. Of the two remaining hybridomas, one responded to both LBB.1 and its transfectant, and the other to neither. RNA was prepared from the four ORF-responsive hybrids with unknown V β s and hybridized with a V β 15-specific probe. All four hybridized with this probe, confirming our suspicion that

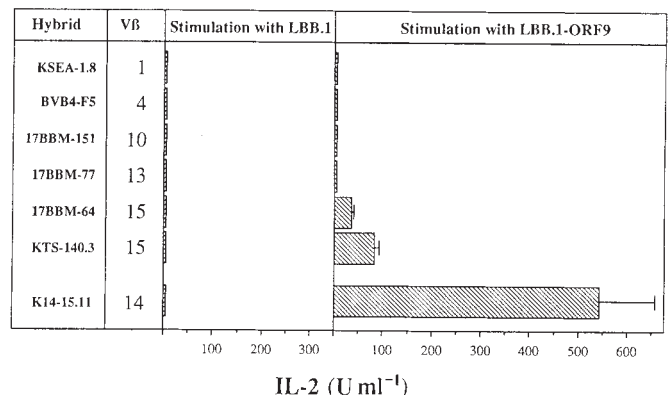


FIG. 3 The product of the C3H exogenous MTV ORF stimulates T cells bearing V β 14 or V β 15. T-cell hybridomas bearing various V β elements were stimulated with LBB.1 or LBB.1-ORF9 and their response measured as previously described^{1,2}. All hybrids were the products of fusion of normal T cells to an $\alpha^- \beta^-$ derivative of the thymoma BW5147⁴⁷ with the exception of hybrids 17BBM-151, 17BBM-77 and 17BBM-64. These hybrids were produced by fusion to BW5147 and so may also bear small levels of TCRs including the endogenous, BW5147-encoded, V β 1⁺ β chain. These three hybrids were the gift of J. Bill and E. Palmer⁵¹. V β expression by the T-cell hybrids was established by dot-blotting with a collection of V β -specific probes.

TABLE 2 Specificity of T-cell hybridomas produced from LBB.1-ORF9-stimulated T cells

T cells bearing V β 14			T cells bearing other V β s			
T cell	IL-2 produced (U ml ⁻¹) in response to:		T cell	V β	IL-2 produced (U ml ⁻¹) in response to:	
	LBB.1	LBB.1-ORF9			LBB.1	LBB.1-ORF9
KOX-10	<10	48	KOX-8	15	<10	209
KOX-31	<10	50	KOX-11	15	<10	197
KOX-32	<10	149	KOX-23	15	<10	155
KOX-44	<10	197	KOX-49	15	<10	168
KOX-48	<10	133				
KOX-49	<10	168	KOX-42	?	232	141
KOX-62	<10	175	KOX-58	?	<10	<10
KOX-69	<10	<7				
KOX-71	<10	20				

CBA/J T-cell blasts produced *in vitro* in response to LBB.1-ORF-9 (Table 1) were fused to the α - β derivative of BW5147 to produce T-cell hybridomas²⁴⁷. Individual hybridomas were stained with anti-V β 14 antibody, 14-2⁴⁸ or the anti-V β 7 antibody, TR 130 (gift of C. Okada and I. Weissman). Representative hybridomas bearing V β 14 and unknown V β s were tested for response to LBB.1 and LBB.1-ORF9. RNA was isolated from the four hybridomas bearing unknown V β s which responded to LBB.1-ORF9 but not LBB.1 and shown to hybridize with a V β 15 probe, confirming that these hybrids bore V β 15.

the ORF-encoded vSAG stimulated V β 15⁺ as well as V β 14⁺ T cells.

Cellular function of ORF product presentation?

In a final set of experiments the ORF gene was transfected into two other B-cell lines, a B-cell lymphoma, CH12.1 (ref. 25), and a B-cell lymphoma hybridoma, LK-4.5 (ref. 26). These were compared with ORF-transfected LBB.1 for presentation of the vSAG using representative V β 14⁺ and V β 15⁺ hybridomas. The results are shown in Fig. 4. As shown before, LBB.1-ORF9 presented the vSAG well to both V β 14⁺ and V β 15⁺ hybridomas. Surprisingly, the other recipients were not as effective. The CH12.1-transfectant presented the vSAG to V β 15⁺ hybridomas, but only very weakly to one of the V β 14⁺ hybridomas. None of the hybridomas responded to ORF-transfected LK-4.5. These results indicated that B-cell lines differed in their ability to present the ORF-encoded vSAG and that V β 15⁺ T cells were more sensitive to stimulation by this antigen than were V β 14⁺ T cells.

To rule out the trivial possibility that the ORF construct was

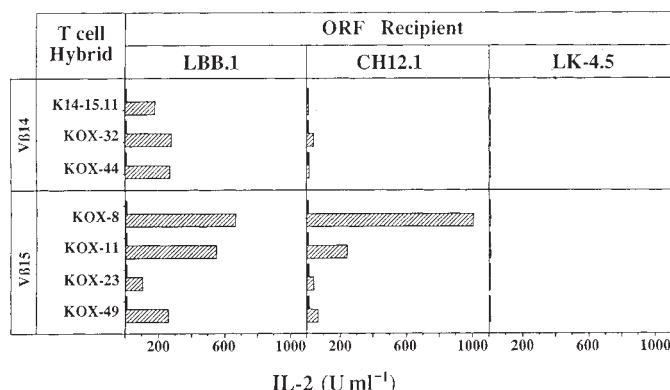


FIG. 4 Differential presentation of the ORF-encoded vSAG by different B-cell lines. The ORF gene construct (Fig. 1) was transfected into the B-cell lymphoma, CH12.1, and into a B-cell lymphoma hybridoma, LK-4.5, for comparison with LBB.1-ORF9. Untransfected recipients (solid bars) and ORF-transfected recipients, LBB.1-ORF9, CH12-ORF9 and LK4.5-ORF3, respectively (hatched bars), were tested for stimulation of representative V β 14⁺ and V β 15⁺ T-cell hybridomas.

poorly transcribed in the second two B-cell lines, messenger RNA from a set of the transfectants was probed for the presence of the 3' untranslated sequence unique to the transfected ORF (C β) and, as a control, for glyceraldehyde 3-phosphate dehydrogenase mRNA (Fig. 5). A probe for ORF itself could not be used diagnostically because the B-cell lymphomas and hybridomas contain ORF mRNA transcribed from mouse endogenous MTVs²³. The untransfected lines contained no transcripts that could hybridize to C β , but three of the four LBB.1-transfectants did. In this cell line the amount of transcript was proportional to the efficiency with which each of these transfectants could stimulate the V β 14⁺ T-cell hybridoma (Fig. 2). LBB.1-ORF9 had the highest amount of transcript and LBB.1-ORF1 had none. Both the CH12.1- and LK4.5-transfectants also contained high levels of C β hybridizing mRNA and therefore presumably also contained high levels of mRNA for the transfected ORF. Variations in ORF mRNA expression, therefore, did not seem to account for the poorer presentation of the vSAG by these two cell lines. These results indicate that LBB.1 may contain high amounts of an activity required for effective presentation of the ORF product, an activity which is limiting in CH12.1 and especially low in LK-4.5. This activity does not seem to be due to MHC class II molecules, because cells of all three of these lines have very high surface levels of these proteins. Significantly, LBB.1 (a hybrid between normal cells from the RF/J mice and a BALB/c B-cell lymphoma) was originally selected for presentation *in vitro* of the MTV-7-associated superantigen, Mls-1⁸ (ref. 24). Perhaps this line has been selected for high quantities of some cellular activity required for processing or transport of the primary vSAG product before it can be effectively presented on the cell surface associated with class II MHC.

Structure of ORF-encoded vSAG

These experiments are the first in which a viral gene encoding a superantigen has been identified. Given that nearly all of the endogenous mouse superantigens have been associated with proviral integrants of MTV^{4,12-16}, it is likely that the 3'LTR ORFs of these proviruses encode superantigens as well. Because each of the known murine superantigens has a particular V β specificity, it is worth examining the known MTV ORFs for any relationship between predicted protein sequence and V β specificity. Complete or partial sequences are available for the ORFs of five MTVs associated with known superantigens^{4,10,14-16,18,20-23}. These are shown in Fig. 6. Overall the predicted ORF protein sequences are very similar with only 10–15% variation. But the sequences of ORF form MTV-9, MTV-8 and

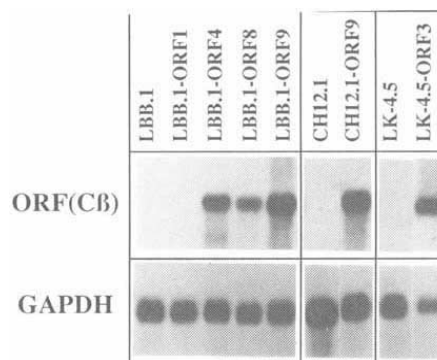


FIG. 5 Northern blot analysis of B-cell transfectants. Poly(A)⁺ RNA was purified from untransfected B-cell lines and their ORF transfected derivatives and analysed by northern blotting as previously described⁴⁹. Messenger RNA transcribed from the transfected ORF was detected using a mouse T-cell-receptor C β probe, which detects the 3' untranslated region of the mRNA. After analysis the filter was washed and hybridized with a GAPDH probe⁵⁰ to control for the amount of mRNA loaded from each cell line. Abbreviations: ORF(C β), MTV LTR ORF/mouse C β 2 fusion mRNA hybridized with a mouse C β probe; GAPDH, glyceraldehyde 3-phosphate dehydrogenase mRNA hybridized with a GAPDH probe.

MTV-11, all associated with deletion of Vβ11-bearing T cells^{4,15,16}, are very similar to each other in the C-terminal region, but quite different from the sequence of MTV-1 (Vβ3-associated¹⁵) and that of the milk-borne virus (Vβ14/15-associated¹¹) studied here. This suggests that the C-terminal region may control Vβ specificity.

The results described here indicate that the Vβ-specific superantigen is encoded by the MTV LTR ORF. But something more complicated could be occurring. For example, the MTV ORF might encode a regulatory protein which induces expression of the Vβ-specific superantigen. If so, the ORFs from different MTVs must encode factors that could each induce a different Vβ-specific superantigen. Although such an idea is formally possible, we think it unlikely and prefer the more straightforward explanation.

Because the product of the ORF gene has never been detected *in vivo*, one cannot be sure at which methionine codon translation starts. The longest protein that can be encoded by the gene has *M_r* ~37K; but other start sites would encode proteins of, for example, about 33, 23, 21 and 19K (Fig. 6). Furthermore, it is not known whether the ORF gene product requires the type of post-translational processing that occurs with products of the *gag* and *env* genes^{17,27,28}. Translation *in vitro* of the ORF gene yields proteins of a variety of *M_s*^{29,30}, but their start and stop sites have not been established. It is presumed that for recognition by T cells of a molecule, the molecule must be expressed on the cell surface: the relatively hydrophobic region after the second methionine could be a signal peptide (Fig. 6). The differences in presentation of the ORF product after transfection of the ORF gene into LBB.1, CH12.1 and LK-4.5 that we found, may indicate a post-translational processing or transport step performed most efficiently in LBB.1.

Biological roles for vSAGs

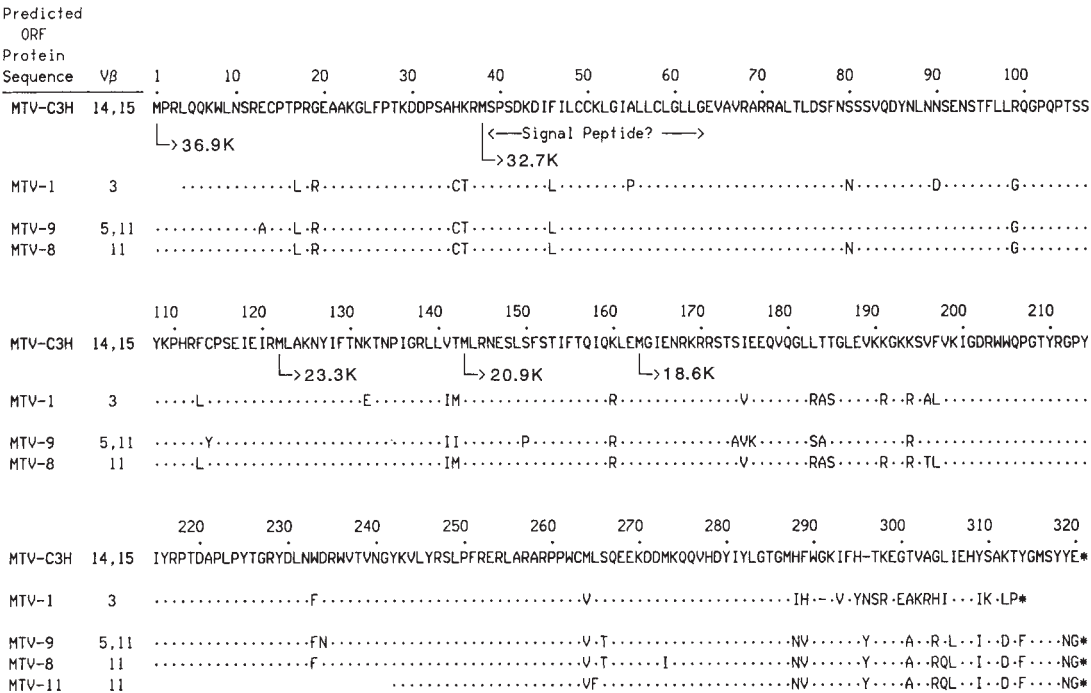
The conserved 3' LTR ORF of MTV has puzzled virologists for some years. Although the LTR contains sequences controlling viral expression^{19,31-33} and various proteins, sometimes with contradictory activities, have been proposed for the product of the open reading frame^{34,35}, no direct evidence of its function has been obtained. Our results suggest that the function of the protein for the exogenous virus may be to act as a superantigen. After infection, ORF expression by the virus could lead to

massive T-cell stimulation. These T cells themselves³⁶, or by-stander cells activated by T-cell products, could then act as reservoirs for the virus which cannot infect the target tissue with which it is most associated, mammary epithelium, until lactation begins. Indeed, the induction of mammary tumours in mice by exogenous MTV is inhibited by neonatal thymectomy^{37,38}. We therefore propose the name vSAG for this gene: 'SAG' for superantigen and 'v' to indicate its viral origin, distinguishing it from bacterial and mycoplasmal SAGs^{39,40}. The vSAG studied here could be called vSAG-C3H to indicate the mouse strain of origin of the virus.

The finding that a wide variety of microbial species produce superantigens leaves open the question of what advantage it can be to the microbe to produce proteins which are such powerful stimulators of the immune response. We have already noted a possible use of a superantigen to MTV, which like other retro-viruses depends on activated cells for infection. It will be important to determine whether other viruses, especially those linked to lymphoproliferative disorders, also use superantigens as a method of gaining a foothold. As far as bacterial SAGs are concerned, the massive production of inflammatory lymphokines induced by these antigens could lead to temporary immunosuppression and hence contribute to infection by superantigen-producing bacteria, such as staphylococci and streptococci^{40,41}. Immunosuppression has also been suggested by the recent evidence that *in vivo* SAGs can induce anergy in T cells bearing target Vβ elements^{42,43}. Finally, it is worth noting that infection of mice with the exogenous C3H MTV leads to a slow disappearance of the target Vβ14-bearing T cells¹¹. This is reminiscent of the depletion of CD4⁺ T cells in human immunodeficiency virus infection.

Although the vSAG studied here is produced by an infectious virus, the other MTV-associated SAGs described so far are associated with genomic proviral MTVs, which rarely, if ever, give rise to a functional virus. These SAGs have considerable effects on the T-cell repertoire, because their presence leads to large deletions of T cells bearing the appropriate Vβ elements. These genomic superantigens are rampant in wild mice⁴⁴. In combination with genetic deletions and inactivations of Vβ genes they lead to the expression of all or most of the Vβ elements in a mouse deme as a whole, but of only a subset of the possible Vβs in mature T cells of individual mice. Why do

FIG. 6 A comparison of the amino-acid sequences (single-letter code) of MTV ORFs with predictable Vβ specificities. ORF sequences were derived from refs 18 and 20-23. The predicted Vβ specificities of each ORF product is derived from refs 4 and 10-16. Asterisks indicate termination codons.



these genetic factors which sharply limit the T-cell repertoire survive evolutionary pressure? We have argued that this situation balances the advantages of a large T-cell repertoire against the disadvantage of offering too many possible targets for microbial superantigens. If the genomic superantigens that cause V β -

specific T-cell deletion are indeed encoded in MTV ORFs, then it seems ironic that the mouse may have co-opted these genes to protect itself both from infection by exogenous viruses using the same superantigen and from bacterial and mycoplasmal attack. □

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Clonal deletion of V β 14-bearing T cells in mice transgenic for mammary tumour virus

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Autoreactive T lymphocytes are clonally deleted during maturation in the thymus. Deletion of T cells expressing particular receptor V β elements is controlled by poorly defined autosomal dominant genes. A gene has now been identified by expression of transgenes in mice which causes deletion of V β 14⁺ T cells. The gene lies in the open reading frame of the long terminal repeat of the mouse mammary tumour virus.

THE thymus plays an important part in the selection of the T-cell repertoire. During maturation, autoreactive T lymphocytes are eliminated and cells with a weak affinity for self major histocompatibility complex (MHC) antigens are positively selected^{1–8}. Clonal elimination of T lymphocytes express-

ing specific T-cell receptor (TCR) V β chains in mice depends on the expression of poorly defined autosomal dominant genes^{1–4,9–13}. The prototype genes responsible for this effect encode the so-called 'minor lymphocyte stimulatory' (Mls) antigens that induce a vigorous primary mixed-lymphocyte reaction^{10,14}. Mls antigens are presented by B cells in association with MHC class II molecules, whereas other cells that express class II molecules apparently cannot present Mls^{15,16}. As well as the classical Mls antigens (Mls-1 to Mls-4), several other structures presented by B cells have been recently described which can induce clonal deletion of T cells expressing specific V β elements^{1,2,11,12,17,18}. None of these structures has been accessible to biochemical analysis.

The discovery that bacterial exotoxins exert similar effects as Mls antigens on the immune system has stimulated interest in these proteins^{19,20}. The enterotoxins of *Staphylococcus aureus* bind very strongly to class II molecules in man and mouse, and expression of class II is required for their presentation²¹. These enterotoxins preferentially stimulate T cells expressing specific TCR V β chains²⁰. Furthermore, when injected neonatally they induce V β -specific clonal deletion²⁰.

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The genetic element responsible for clonal deletion of T cells bearing V β 5 and V β 11 is tightly linked to *Mtv-9*, an endogenous mouse mammary tumour virus (MMTV) proviral locus²². In the genome of laboratory mice, different subsets of the 20–30 endogenous MMTV proviral loci are stably integrated²³. Their gene products differ by 5–10% in amino-acid sequence. To attribute directly a role to MMTV gene products in the clonal deletion of autoreactive cells, we analysed lines of transgenic mice bearing either the complete exogenous provirus MMTV (GR) or only the open reading frame (*orf*) present in the long terminal repeat (LTR) of the provirus. These mice were screened for deletion of T cells expressing any of the TCR V β regions detectable with the currently available monoclonal antibodies. A complete and specific deletion of V β 14-expressing T cells was found in the periphery of transgenic mice bearing either the whole MMTV (GR) provirus or only the *orf* (GR) gene. This deletion of V β 14-expressing cells was strictly dependent on class II I-E expression.

Genetic analysis

Mls phenotype and MMTV genotype were initially compared in 18 different mouse strains. Results from the literature as well as our own results were included in this comparison (data not shown)^{23–25}. In all the cases a strict correlation between *Mtv-6* expression and the Mls-3^a phenotype and *Mtv-7* expression and the Mls-1^a phenotype was observed.

Clonal deletion in MMTV (GR) transgenic mice

To directly test the hypothesis that MMTV expression leads to clonal deletion of T cells expressing particular TCR V β elements, we analysed transgenic mice that contain the entire MMTV (GR) genome²⁶. MMTV (GR) is the product of *Mtv-2*, a recently acquired, spontaneously activated provirus of GR mice responsible for high tumour incidence²⁷. The DNA used for generating these mice is shown in Fig. 1a. MMTV expression

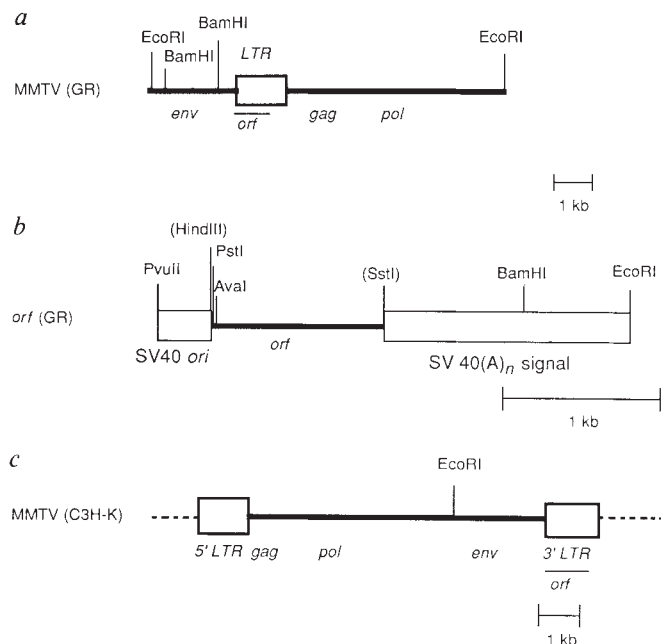


FIG. 1 DNA fragments used for generation of transgenic mice. *a*, MMTV (GR), driven by its LTR. The DNA was cloned from unintegrated circular DNA of exogenous virus and the circle was opened with *EcoRI* digestion. The linearized fragments were ligated with T4-DNA ligase to reconstruct a complete provirus by head-to-tail ligation. *b*, The *orf* of MMTV (GR) controlled by SV40 promoter and poly(A) addition signals. *c*, MMTV (C3H-K), driven by its own LTR. MMTV (C3H-K) transgenic mice contain a short sequence of cellular DNA at either end as indicated by the dotted lines. The fragments were cloned as described^{26,37,48}. The restriction sites for construction are indicated.

TABLE 1 Clonal deletion of V β 14⁺ T cells in MMTV transgenic mice

Mouse line	Number of mice	I-E expression	V β 8 (percentage of CD4 ⁺)	V β 11 (percentage of CD4 ⁺)	V β 14 (percentage of CD4 ⁺)
MMTV (GR) A	6	+	24.6 $\pm$ 1.5	0.7 $\pm$ 0.3	0.3 $\pm$ 0.4
MMTV (GR) A	3	–	15.8 $\pm$ 2.6	2.8 $\pm$ 0.8	6.8 $\pm$ 0.7
MMTV (GR) B	2	+	24.2 $\pm$ 2.2	0.9 $\pm$ 0.2	0.1 $\pm$ 0.1
MMTV (GR) B	3	–	18.5 $\pm$ 0.3	3.7 $\pm$ 0.1	7.2 $\pm$ 0.5
MMTV (GR) C	3	+	16.7 $\pm$ 3.5	0.5 $\pm$ 0.3	0.8 $\pm$ 0.0
MMTV (GR) E	3	+	20.4 $\pm$ 5.3	0.3 $\pm$ 0.2	0.6 $\pm$ 0.3
MMTV (GR) F	3	+	26.4 $\pm$ 3.5	0.9 $\pm$ 0.7	1.3 $\pm$ 0.6
MMTV (GR) G	3	+	20.9 $\pm$ 7.2	0.3 $\pm$ 0.3	0.4 $\pm$ 0.2
Controls	4	+	19.6 $\pm$ 1.6	1.3 $\pm$ 0.4	7.2 $\pm$ 1.4
MMTV (GR) orfII	1	+	ND	ND	1.6
MMTV (GR) orfIII	5	+	23.6 $\pm$ 6.0	ND	2.2 $\pm$ 1.4
MMTV (GR) orfIV	9	+	18.7 $\pm$ 6.0	ND	0.6 $\pm$ 0.5
Orf controls	12	+	17.3 $\pm$ 6.1	ND	9.0 $\pm$ 0.7
MMTV (C3H-K) X	5	+	23.5 $\pm$ 2.1	0.6 $\pm$ 0.2	9.1 $\pm$ 0.5
MMTV (C3H-K) W	5	+	9.4 $\pm$ 0.5	0.8 $\pm$ 0.3	10.2 $\pm$ 1.1
BALB/c	3	+	18.4 $\pm$ 0.3	0.5 $\pm$ 0.5	8.1 $\pm$ 1.8

Lymph node cells of male and female mice older than 8 weeks were analysed. MMTV (GR) A, B, C, E, F and G are independent MMTV (GR) transgenic mouse lines. MMTV (C3H-K) W and X are independent transgenic mice with MMTV (C3H-K). MMTV (GR) orfII, orfIII and orfIV mice are three independent transgenic mouse lines for the *orf* sequence of MMTV (GR). In the case of *orf* transgenic mice, peripheral blood of 8-week-old mice was analysed. Controls were nontransgenic littermates. Variations in the V β 8 staining can be explained by heterozygosity at the TCR β -chain locus. V β 8 is genomically deleted in SJL mice⁵¹. Heterozygous mice express about 50% of normal V β 8 levels. ND, not determined. Mice used for fluorescence-activated cell sorting (FACS) analysis were descendants of the original (C57BL/6 $\times$ SJL)_{F2} transgenic founder mice crossed once with BALB/c mice and then maintained as brother-sister matings. Lymph node cells (10⁶) were stained with the anti-TCR V β -specific monoclonal hybridoma supernatants KJ-16-133 (anti-V β 8.1 and 8.2)⁵⁰, RR3-15 (anti-V β 11)¹⁴ and 14.2 (anti-V β 14)²⁸ and then stained with fluorescein-conjugated goat anti-rat IgG or IgM antisera. In the second dimension, phycoerythrin-coupled anti-CD4 monoclonal antibody GK1.5 was used. Dead cells were gated by a combination of forward and perpendicular light scatter. Background-staining values obtained with the second-stage reagents alone were subtracted. Analysis was performed on a FacScan (Becton-Dickinson) cell analyser.

is driven by its own LTR. S1-nuclease mapping of RNA of transgenic animals revealed high expression of MMTV in the mammary gland, intermediate expression in the salivary gland, low expression in spleen and low to undetectable expression in other organs (Fig. 2a).

Lymph node cells of transgenic mice, nontransgenic littermates or control BALB/c mice were tested for elimination of T cells bearing a specific TCR V β element. A dramatic (≥ 10 -fold) deletion of CD4⁺ T cells expressing TCR V β 14 was detected in the transgenic mice (Table 1). Numbers of cells expressing both CD8 and V β 14 are very low in mice not expressing H-2^k, owing to lack of positive selection²⁸, and hence these cells were not analysed further. None of the other TCR elements tested (V β 2, 3, 4, 5, 6, 7, 8, 9, 11, 13) was reduced in the CD4⁺ subset as a result of MMTV (GR) transgene expression (data not shown). V β 14 deletion in MMTV (GR) transgenic mice was independent of the integration site and number of copies of the transgene. The same deletion patterns were found in six independent transgenic mouse lines with copy numbers ranging from 6 to 20. Therefore it seems unlikely that clonal elimination is induced through activation of cellular genes in *cis* through the MMTV LTR, as observed in MMTV-induced mammary tumours^{29,30}.

Most V β deletion elements are strictly dependent on class II I-E expression to induce clonal deletion. Such deletion elements include those for V β 5, 11, 12, 16 and 17. In the MMTV (GR) transgenic mice we found that I-E expression is required for the clonal deletion, because transgenic animals not expressing I-E show comparable levels of peripheral V β 14 expression to nontransgenic (I-E-positive) littermates (Table 1).

The kinetics of clonal deletion are remarkably slow. Complete deletion of V β 14⁺ cells in MMTV (GR) transgenic mice is not observed until 4–5 months of age (Fig. 3). In contrast, the kinetics of clonal deletion of V β 3 and V β 6 in mice expressing both Mls-1^a and -3^a is much more rapid, reaching completion within

the first 10 days after birth^{31,32}. Interestingly, both the V β 3 and V β 6 deletion elements (defined originally as MIs antigens) stimulate a vigorous primary mixed lymphocyte reaction, whereas the deletion elements for V β 14 (data not shown) as well as V β 5, 11, 12, 16 and 17 do not stimulate (or only stimulate weakly) a primary mixed lymphocyte reaction. In addition, the first two are presented by certain I-A alleles as well as I-E, whereas the latter are presented exclusively by I-E. It is possible that strong stimulation *in vitro* (and rapid deletion *in vivo*) of V β 3⁺ and V β 6⁺ T cells results from a higher-affinity interaction with the corresponding class II/MIs antigen complex.

Clonal deletion in *orf* (GR) transgenic mice

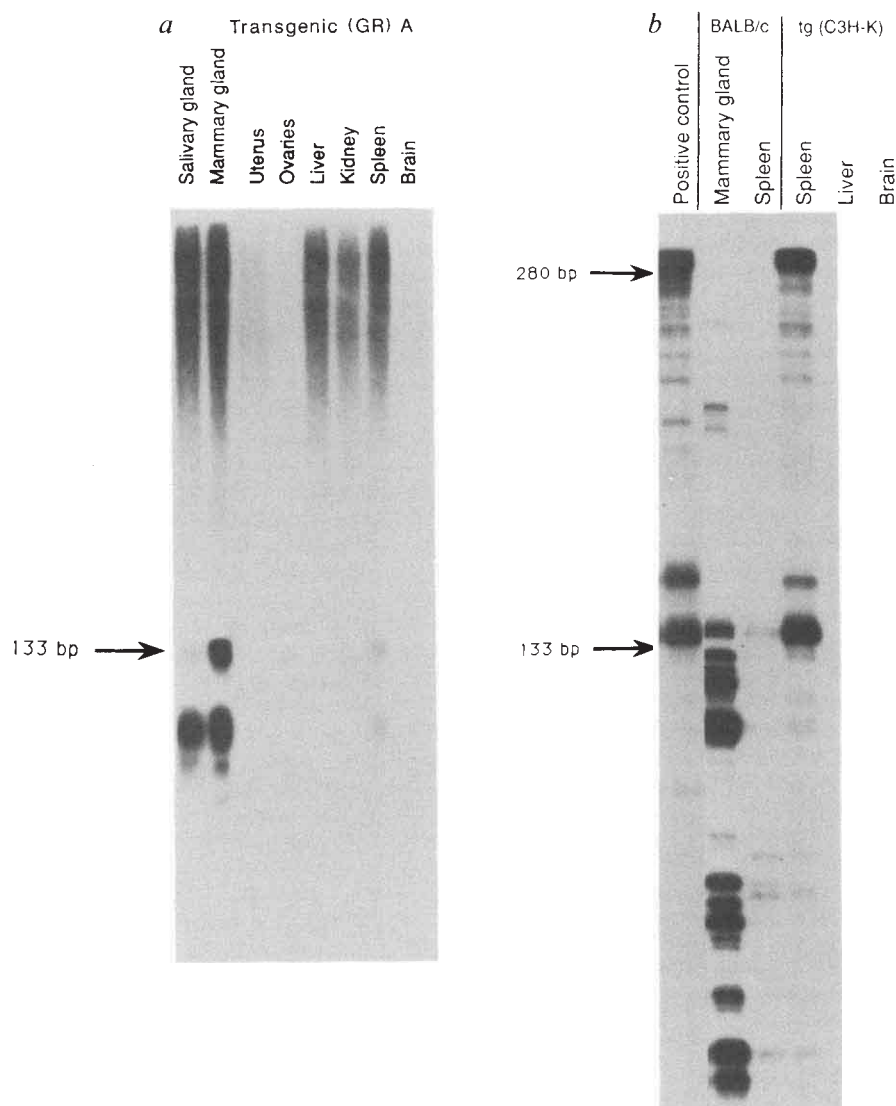
MMTV has the normal genetic constitution of a non-acutely transforming retrovirus coding for Gag, Pol and Env proteins. But it contains an unusually long U3 region in its LTR. The ~960-base-pair (bp) *orf* can encode a polypeptide of relative molecular mass ~36,000 ($M_r \sim 36$ K), whose function is still controversial^{33,34}. The *orf* is highly conserved between endogenous and exogenous virus genomes, but discrete sequence differences are observed. To test whether the *orf* gene is responsible for clonal deletion of V β 14⁺ T cells, we analysed transgenic mice derived from three independent founder mice, which contain the MMTV (GR) *orf* sequence under the control of the simian virus 40 (SV40) promoter (Fig. 1b). The *orf* (GR)

transgenic mice showed a V β 14 deletion comparable to that in transgenic mice with the whole MMTV (GR) genome (Table 1).

Lack of clonal deletion by a MMTV variant

MMTV of C3H mice which is maternally transmitted through milk, also induces V β 14 T-cell deletion in the periphery³⁵. Proteins encoded in the *orf* of GR and C3H viruses differ by 23 amino acids³⁶. Assuming that the *orf* gene product is responsible for the V β 14 deletion in both instances, then the differences in 23 of the 320 amino acids (Fig. 4) might not affect the deletion patterns. We therefore analysed transgenic mice expressing a variant of MMTV (C3H) with different biological properties (Fig. 1c). The mutant virus MMTV (C3H-K) was isolated from a kidney adenocarcinoma and no longer induces mammary tumours³⁷. The kidney-specific proviral DNA contains several point mutations compared with MMTV (C3H). The main difference, however, is a substitution of LTR sequences resulting in the replacement of amino acids 288–315 and a deletion of the last four amino acids of the *orf* coding region (Fig. 4). These transgenic mice do not delete V β 14⁺ T cells (Table 1). If *orf* proteins really are responsible for the MMTV (C3H)-induced V β 14 deletion, then the C-terminal 32 amino acids could play a crucial role. As for the full MMTV (GR) transgene, expression of MMTV (C3H-K) genes was detected in spleen (Fig. 2b). The transgenic line MMTV (C3H-K) X shows high expression of

FIG. 2 *a*, S1-nuclease protection assay of RNA extracted from different organs of a lactating transgenic MMTV (GR) female. The localization of the protected 133-bp band is indicated. *b*, Transcription of the MMTV provirus in a transgenic female mouse of the MMTV (C3H-K) X-line. Expression was analysed with an RNase protection assay that distinguishes between transcription of the transgene and the endogenous copies of MMTV. Because of the sequence substitution in the U3 region of MMTV (C3H-K)³⁷, the protected 280-nucleotide fragment is specific for MMTV (C3H-K) transcription. RNA from a serially transplanted kidney tumour was used as a positive control. This tumour, SC7, expresses high levels of MMTV (C3H-K) RNA. For comparison, the expression of endogenous MMTV RNA in BALB/c mice (lactating mammary gland and spleen) is shown. We used 20 μ g total RNA from BALB/c spleen, transgenic (C3H-K) X spleen, liver, brain and kidney, 10 μ g of total RNA from BALB/c mammary gland, and 0.5 μ g total RNA from SC7 cells. METHODS. *a*, The *Pst*I–*Pvu*II fragment of the MMTV (GR) DNA was 5'-labelled and hybridized to 50 μ g cellular RNA. After treatment of the hybrids with 1,000 U ml⁻¹ S1 nuclease, the protected DNA fragments were separated on a 6% polyacrylamide-urea sequencing gel. The fragment of 133 nucleotides corresponds to correctly initiated viral RNA. *b*, The *Pst*I–*Bam*HI fragment of the 3' LTR of the exogenous MMTV (C3H-K) provirus, containing about 30 bp of adjacent cellular sequences, was cloned in the SP65 vector, and linearized with *Dra*I before transcription *in vitro* according to the procedure described⁴⁹, using 0.5–20 μ g total cellular RNA per assay.



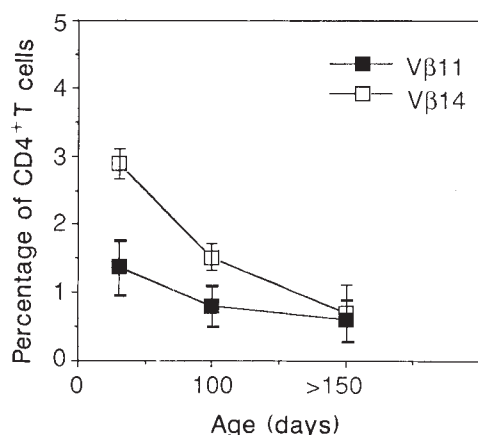


FIG. 3 Ontogeny of TCR V β 14 deletion in MMTV (GR) transgenic mice. Lymph-node cells from mice of different ages were analysed as described in the legend to Table 1 for expression of TCR V β 11 and V β 14. At least five mice were analysed at each time point and indicated as mean $\pm$ s.d.

the transgene in the spleen, whereas for the line MMTV (C3H-K) W, expression is low but detectable.

Conclusions

Because of the strong genetic correlation between MMTV expression and MIs phenotype we analysed transgenic mice that harbour several copies of MMTV (GR) DNA, and found that these mice have clonally deleted peripheral T cells expressing V β 14. Transgenic mice with only the *orf* gene located in the 3' LTR of this virus showed the same pattern of deletion. Clonal deletion of V β 14⁺ T cells was strictly dependent on I-E, as transgenic mice that do not express I-E had the same numbers of V β 14-bearing T cells as nontransgenic littermates that express I-E. Together our data indicate that the *orf* gene product acts as an MIs-like structure.

There are at least four main possibilities for the mechanism of action of *orf* proteins: (1) they could activate a gene *in trans* to induce clonal deletion; (2) they could modify a different protein to induce clonal deletion; (3) they could act like bacterial enterotoxins by crosslinking TCR and class II molecules; or (4) processed *orf* peptides could interact with class II molecules and induce clonal deletion.

Although there is so far no direct evidence, the first two possibilities seem unlikely. At least 10 specific but distinct V β T-cell deletion patterns have been observed with endogenous deletion elements. If most or all of the deletion elements were *orf*-encoded molecules, the 5–10% disparity between the different *orf* amino-acid sequences must be sufficient to regulate other genes or proteins so as to accommodate the above results. It seems more likely to us that the *orf* proteins interact directly with class II molecules. The experiments described here cannot address whether the *orf* gene product acts as an intact molecule or in the form of a processed peptide being presented by class II. In either case, it is noteworthy that most of the sequence variation between *orf* proteins lies in the C-terminal end^{36–40}.

The *orf* gene of MMTV has long puzzled retrovirologists. It is found in nearly all the reported MMTVs. Low amounts of *orf* messenger RNA are expressed and it has so far been impossible to detect the *orf* protein in infected cells or primary mammary tumours. The *orf* protein has been proposed to act as a transcriptional activator³³ or a transcriptional repressor³⁴. On the basis of our data, one of the functions of *orf* could be to enable MMTV to take advantage of the immune system. Because the normal route of infection of MMTV is by uptake of milk

ORF (GR)	-	MPRLQQKWLNSRECPTRLGEAAKGLFPTKDDPSAHKRMSPSDKDILILCC	-50
ORF (C3H)	-	-----P-----V-----F-----	-50
ORF (C3H-K)	-	-----Q-----G-----	-50
ORF (GR)	-	KLGIALLCGLLGEVAVRARRALTDSFNNSVQDYNLNDSNSTFLLG	-100
ORF (C3H)	-	-----S-----*S-----N-----R-----	-99
ORF (C3H-K)	-	-----S-----*S-----N-----R-----	-99
ORF (GR)	-	QGQPQTSYKPHRICPLEIEIRMLAKKYIFTNKTNPIGRLLVTLRNESL	-150
ORF (C3H)	-	-----F-----S-----N-----T-----N-----	-149
ORF (C3H-K)	-	-----L-----S-----N-----T-----N-----	-149
ORF (GR)	-	SFSTIFTQIQKLEMGIEENKRRSKSIEEQVQGLLAGLEVKKGKSVFVK	-200
ORF (C3H)	-	-----T-----V-----R-----AL-----	-199
ORF (C3H-K)	-	-----R-----T-----V-----R-----AL-----	-199
ORF (GR)	-	IGDRWWQPRTYRGPYIYRPTDAPLPYTRYDNLNDRWVTINGYKVLRYSL	-250
ORF (C3H)	-	-----LG-----V-----	-249
ORF (C3H-K)	-	-----G-----V-----	-249
ORF (GR)	-	PFERLARARPPWCMLTEKEKDDMKQVVDYIYLGTGMHFWGKVFHTKEG	-300
ORF (C3H)	-	-----S-----S-----I-----	-299
ORF (C3H-K)	-	-----V-----S-----V-----R-----RDLNVE-KSR-E	-299
ORF (GR)	-	AVAGLIEHYSAKTYGMSYYD	-320
ORF (C3H)	-	-----P-----E-----	-319
ORF (C3H-K)	-	VQKH--D**--I--ALPL--	-315

FIG. 4 Amino-acid sequences (single-letter code) of the *orf* proteins of MMTV (GR), MMTV (C3H) and the mutant virus MMTV (C3H-K). The MMTV (C3H-K) sequence is the corrected version of the sequence of ref. 37.

through the gut and infection of local lymphocytes⁴¹, the presence of *orf* protein might favour the stimulation of infected B cells by T cells expressing a specific TCR V β element. At the same time, infected or noninfected T cells could be stimulated. As stimulation triggers proliferation, this could facilitate integration of the newly synthesized provirus, and at the same time allow expansion of infected cells. This would be an advantage for the survival of the virus.

Although the present data directly establish only exogenous MMTV (GR) as a V β 14 deletion element, it seems likely that most (if not all) V β deletions in the mouse are mediated by a similar mechanism^{35,42–44}. If generally true, then the identification of endogenous MMTV proviral loci as V β deletion elements could explain several puzzling properties of these MIs-like structures, including: (1) the behaviour as autosomal dominant genes; (2) the existence of both stimulatory and null 'alleles' (resulting from random segregation of proviral integration sites); (3) the apparent intercellular transfer of MIs products in radiation bone marrow chimaeras^{12,45} (perhaps due to radiation-induced expression of infectious proviral particles); and (4) the failure so far to observe V β -specific deletions in other species such as rat and man.

The retroviral genes described here could have considerable effects on the immune system, because they can mediate immune stimulation^{10,14} and induce anergy^{46,47} as well as deletion. It remains to be established whether ectopic expression of such genes, after tolerance towards self-antigens has been established, triggers organ-specific inflammation and autoimmunity. □

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LETTERS TO NATURE

An estimate of the Hubble constant from the gravitational lensing of quasar Q0957+561

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THE double quasar Q0957+561 AB is believed to be a gravitationally lensed image formed by a galaxy at redshift $z=0.36$: a single quasar at $z=1.41$ is seen as two images because of the intervening galaxy. I present here a measurement of the velocity dispersion of the intervening galaxy which, combined with a measurement of the time delay between the appearance of brightness variations in the two images, can be applied to a simple model of the lensing geometry to yield an estimate for the Hubble constant H_0 . The flux from the A and B images has been monitored for several years by two groups and a consistent value of 415 days for the time delay between variations in A and B has been obtained. The measured line-of-sight velocity dispersion for the intervening galaxy of $303 \pm 50 \text{ km s}^{-1}$ then gives $H_0 = 50 \pm 17 \text{ km s}^{-1} \text{ Mpc}^{-1}$. Q0957+561 AB was the first example of a lensed quasar to be discovered¹. This quasar is believed to be a gravitationally lensed image formed by a galaxy at a redshift of 0.36 (ref. 2). Deep images² reveal a cluster of galaxies whose brightest member (the deflector) is nearly superimposed on Q0957+561B. Multichannel scans of the quasar show a contribution from this galaxy, and the location of the 4,000-Å break in the galaxy spectrum indicates a redshift of 0.36 (ref. 9). Images taken with 0.3–0.5-arcsec seeing³ confirm that the galaxy is 1.0 ± 0.03 arcsec north and 0.24 ± 0.09 arcsec east of the southern quasar image (B). The system has been imaged in the infrared (K-band)⁴, where it is found that the brightness profile of the galaxy matches that of a cD galaxy (giant elliptical with an extended halo). The galaxy has an apparent magnitude of 18.49 ± 0.03 for a Gunn r filter, a core radius of $0.24'' \pm 0.09''$, and an ellipticity of 0.13 ± 0.01 .

From 1980 onwards, photometric monitoring of the A and B images was carried out by two groups^{5,6}. The quoted time delays are 415 ± 20 days⁵ and 404 ± 10 days⁶. The time delay is such that $\Delta\tau_{BA}$, the difference in the light propagation times from the source to the observer, is positive (image A varies first). These values are based on data acquired independently on different telescopes by different observers and their consistency is evidence that the effect is real. Both groups also suggest that

the variation with time of the true brightness variation between the two images is indicative of microlensing.

Simple models have been developed that account for the known properties of the lens system^{7,8}. The lensing matter is modelled by three components: (1) the bright galaxy; (2) a compact nucleus; and (3) the cluster. The brightest galaxy is represented as a circularly symmetric smooth King¹⁰-type surface density profile whose parameters are the core radius and velocity dispersion. The compact nucleus has one parameter (its mass), and the cluster is modelled by a smooth distribution of dark matter with a given surface density. The compact nucleus is required to account for the relative magnification of the A and B images. The model has fewer parameters than the observations and can account for them well. The time delay is proportional to the product of the distance to the deflector and the distance to the quasar divided by the distance from the deflector to the quasar. As each distance scales as H_0^{-1} , the time delay is inversely proportional to H_0 ; one can therefore use the model above⁸ to obtain bounds on the Hubble constant. The full expression in terms of observable parameters is:

$$H_0 = (90 \pm 10)(\sigma_v/390 \text{ km s}^{-1})^2(\Delta\tau_{BA}/1 \text{ yr})^{-1} \text{ km s}^{-1} \text{ Mpc}^{-1},$$

where σ_v is the velocity dispersion of the brightest galaxy.

Independent of measurement errors in $\Delta\tau_{BA}$ and σ_v , an error of 10% is quoted for the Hubble constant in the above formula. This error estimate accounts for the uncertainty in the model⁸. The sources of this error estimate are: (1) the uncertainty in Ω , the density parameter of the Universe; (2) uncertainties in the relative angular positions of the A and B images and the galaxy

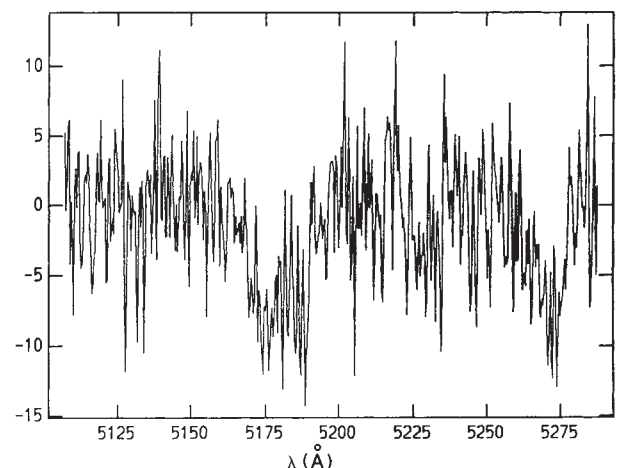


FIG. 1 The galaxy spectrum continuum subtracted and shifted to $Z=0$.

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position; and (3) the range in the model parameters that can be varied to affect H_0 without influencing the other observables in the problem.

Motivated by the unambiguous measurement of $\Delta\tau_{BA}$ mentioned above, I set out to measure the velocity dispersion σ_v of the lensing galaxy and the redshifts of the brightest galaxies in the putative cluster. Measuring the velocity of the brightest galaxy is nontrivial because the galaxy spectrum is seriously contaminated by quasar light: in fact, the 'sky background' is dominated by the continuum emission from the quasar. Observations were carried out on the night of 19/20 February 1990 using the 4.2-m William Herschel Telescope at the Observatorio del Roque de los Muchachos. The ISIS triple spectrograph¹¹ was used at the $f/11$ Cassegrain focus of the William Herschel Telescope. The R600R grating was used with effective blaze at 7,000 Å and a resolution of ~ 1 Å. The wavelength coverage for observing the cD galaxy was 6,600–7,400 Å, chosen so as to include the MgIb triplet at 5,174 Å redshifted at $z = 0.36$.

A $1''$ slit oriented in the north-south direction was centred on Q0957+561B. Five 2,400-s integrations were made with this setup. The five CCD frames were median filtered and wavelength calibration was made using a helium-argon arc exposure. The spectra were corrected for misalignment (the dispersion axis did not coincide exactly with the y -axis on the CCD), and the data were sky subtracted. The reduction was done using the IRAF reduction package. With such a setup the profile of Q0957+561B is asymmetric, the northern wing of the profile being brighter than the southern wing because of the contribution of the cD galaxy light. The columns of the northern wing of the quasar profile were summed so as to give a spectrum that contained the maximum signal-to-noise ratio for the galaxy. The galaxy redshift was measured and the spectrum was de-redshifted. The lines of the MgIb triplet are clearly visible in Fig. 1 at 5,174 Å. The redshift and velocity dispersion were measured using the cross-correlation technique¹². The template spectrum used was a spectrum of the standard star HD207229. This method yields a line-of-sight velocity dispersion of 303 ± 50 km s⁻¹ for the galaxy. Using the simple model described above and assuming the time delay between the two quasar images to be 415 days, the value of H_0 turns out to be 50 ± 17 km s⁻¹ Mpc⁻¹.

A key unknown in this problem is the mass of the cluster and its contribution to the bending. During the same observing run I intended to measure the velocity dispersion of the cluster using 30 galaxies, but because of poor weather only six redshifts of cluster members were obtained. These data yield an r.m.s. line-of-sight velocity dispersion¹³ of 530 km s⁻¹. Redshifts for the galaxies were measured using a standard cross-correlation technique¹² (the template used was M32¹⁴). The uncertainties in this number are large and ~ 30 redshifts of cluster members will be required to assess the contribution of the cluster gravitational potential to the lensing. □

A high-resolution radio image of a young supernova

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SUPERNOVAE in our own Galaxy are so rare that images of their remnants¹ can show only the late aftermath of an explosion that occurred anything from a few hundred to several tens of thousands of years ago. Young supernovae are seen frequently in other galaxies, but because they are more distant, it has not been possible until now to obtain high-resolution images that would reveal details of the explosion and the immediate development of the ejected material. Here we present a very-long-baseline interferometric (VLBI) radio image of the bright supernova 1986J, which occurred in the galaxy NGC891 at a distance of ~ 12 Mpc. No detailed image of any supernova or remnant has been obtained before so soon after the explosion. Our image shows a shell of emission with jet-like protrusions. Their analysis should advance our understanding of the dynamics of the expanding debris, the dissipation of energy into the surrounding circumstellar medium, and the evolution of the supernova into the remnant.

Supernova 1986J, in the galaxy NGC891, is the most luminous radio supernova yet discovered^{2,3}. At 56% (ref. 4) to 68% (ref. 5) of the ~ 20 Mpc^{6,7} distance to the Virgo cluster, SN1986J has a peak luminosity at a frequency of 8.4 GHz which is $\sim 4,000$ times greater than Cas A's at present and several times greater than that of the most luminous supernova or supernova remnant previously known. SN1986J's explosion date is not known precisely, but was estimated from the radio light curves at several wavelengths and the sparse optical data available, to be the end of 1982, with an uncertainty of ~ 8 months⁸ (see also refs 3, 9). The progenitor star probably had a mass of 20–30 $M_\odot$ ^{3,10}. The debris of SN1986J is highly clumped¹⁰, may consist of a mixture of thermal absorbers and non-thermal emitters⁸, and is expanding into a dense circumstellar medium with mean maximum velocity of $\sim 15,000$ km s⁻¹, inferrable from our earlier VLBI size determinations¹¹. These determinations also gave a first indication of SN1986J's brightness distribution, showing that it deviates from spherical symmetry and is elongated along a position angle of $\sim 145^\circ$. The present observations were far more sensitive, involved more telescopes, and provided a higher angular resolution, thus allowing us to make a detailed image of SN1986J.

We made VLBI observations of SN1986J with eight telescopes (see Table 1) at a frequency of 8.4 GHz on 29 September 1988, about six years after the estimated date of explosion. At each station, a hydrogen maser frequency standard was used to govern the local oscillator chain and the 'time tagging' of the recorded data. All observations were made with the Mark III VLBI system¹² in right-hand circular polarization (IAU convention). The data were correlated with the Mark III VLBI processor of the Haystack Observatory and then calibrated in the manner usual for Mark III VLBI data.

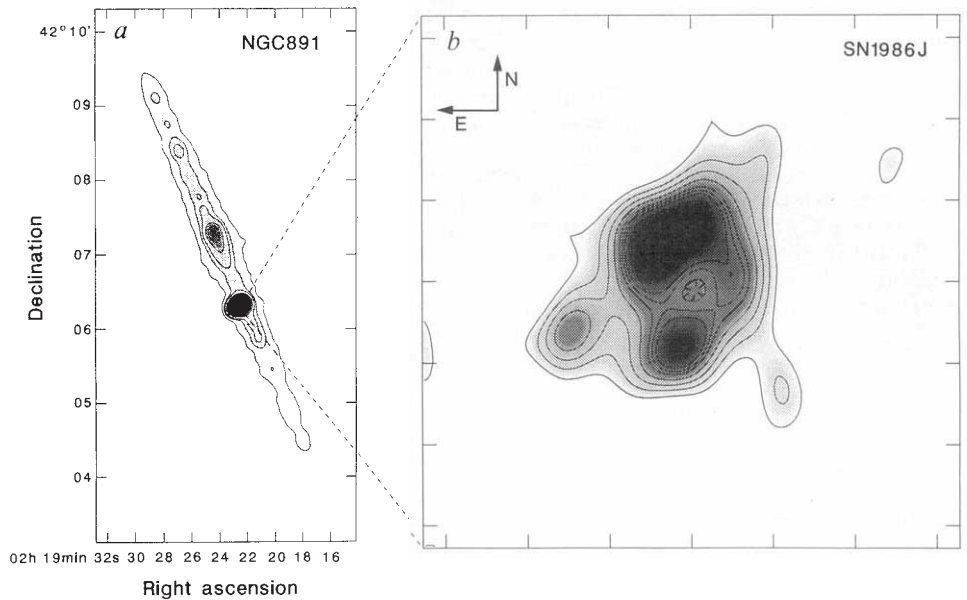
Using the Astronomical Image Processing System of the National Radio Astronomy Observatory, we produced an image of SN1986J with the maximum entropy deconvolution algorithm. Figure 1 shows the image, together with a radio map of the host galaxy, NGC891. The image of SN1986J shows a variety of details. The brightness distribution has the form of a shell, with a local minimum at its centre. The brightness of the rim of the shell has three maxima, the largest located northeast

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FIG. 1 *a*, A 'clean' map of the galaxy NGC891 and its supernova 1986J made with the Very Large Array at the frequency and epoch of the VLBI observations on 29 September 1988. The contours are at $-0.2, +0.2, 0.6, 1.1, 1.7, 2.9, 5.7, 8.6, 11, 29, 57$ and 86% of the peak brightness. The dimensions (full width at half maximum) of the restoring beam are $10.4'' \times 8.4''$, with the beam's major axis orientated along a position angle of -60° . The coordinates are for epoch B1950.0. *b*, A VLBI 'maximum entropy' image of SN1986J at a scale 10^5 times that of *a*. The contours are at 10, 20, 30, 40, 50, 55, 60, 70, 80, 90 and 99% of the peak brightness. The flux density integrated over the map area is 51 mJy . Tick marks are separated by 1 mas—equivalent to $\sim 12,000 \text{ AU}$, or $1.8 \times 10^{17} \text{ cm}$. The effective angular resolution in the image is $\sim 0.6 \text{ mas}$. North is up and east is to the left.



of the centre with a peak brightness temperature of $\sim 3 \times 10^8 \text{ K}$. The most intriguing features discovered were two protrusions (with a possible third in the southwest). They extend from the centre to twice the radius of the shell; their lengths are equal to within 6% of their mean— 1.9 mas at the 20% contour level. Given a distance to NGC891 of $D = 12 \text{ Mpc}$, this mean projected length is equivalent to $\sim 23,000 \text{ AU}$ or $3.4 \times 10^{17} \text{ cm}$, and the mean expansion velocity, projected on the sky, is $\sim 18,000 \text{ km s}^{-1}$, calculated assuming that the protrusions originated in the centre at the time of the explosion. Also, there is evidence, although marginal, that the azimuth of each of the protrusions corresponds to that of a local minimum in the brightness of the rim of the shell. The largest diameter of the supernova at the 20% contour level is 3.7 mas , equivalent to $\sim 44,000 \text{ AU}$, or $6.6 \times 10^{17} \text{ cm}$, given $D = 12 \text{ Mpc}$.

As the radiation is synchrotron, the minimum sum, $E_{\min}^{\text{tot}}$, of the energies in the magnetic fields, the electrons and the heavy particles can be estimated¹³ (assuming equipartition):

$$E_{\min}^{\text{tot}} \approx 3 \times 10^{48} (1+k)^{4/7} \phi^{3/7} (D/12 \text{ Mpc})^{17/7} \text{ erg}$$

where k is the ratio of the total energy of the heavy particles to that of the electrons, and ϕ is the fraction of the supernova's volume occupied by the magnetic field and by the relativistic particles. The value for $E_{\min}^{\text{tot}}$ is attained for the magnetic field, $B_{\min}$:

$$B_{\min} \approx 0.03(1+k)^{2/7} \phi^{-2/7} (D/12 \text{ Mpc})^{-2/7} \text{ G}$$

As $1 \leq k \leq 2 \times 10^3$, $E_{\min}^{\text{tot}}$ can attain values between $4 \times 10^{48} \text{ erg}$ and $2 \times 10^{50} \text{ erg}$ for $\phi = 1$ and $D = 12 \text{ Mpc}$. The corresponding values for $B_{\min}$ range from 0.04 G to 0.3 G . Since $\phi \leq 1$ and, most likely, $D < 18 \text{ Mpc}$, $E_{\min}^{\text{tot}}$ is smaller than the kinetic energy of $\sim 1.5 \times 10^{51} \text{ erg}$ derived for SN1987A (ref. 14), for which the mass of the progenitor was probably about equal to that of the progenitor of SN1986J.

How can the brightness features be interpreted? Our previous observations of the brightness distribution's elongation lead us to consider three interpretations. First, the radio emission could emanate from the relativistic plasma powered by a pulsar^{15,16}, in which case an elongated plerion, a centre-filled brightness distribution, could be expected. Second, the density of the circumstellar medium could vary with azimuth about the centre if the progenitor's wind were not isotropic. When the shock front interacts with this anisotropic medium^{17,18}, the brightness distribution is expected to deviate from circular symmetry. Third, the flow pattern of the expanding debris could itself be anisotropic, caused for instance by the rotation of the progenitor star¹⁹ and/or by an asymmetric explosion process¹⁹, which would also lead to an asymmetric brightness distribution.

The image in Fig. 1 places limits on these interpretations. The indication that the brightness distribution is a shell is inconsistent with a purely plerionic morphology. Also, at the time of the last VLBI observations, the spectral index α between 5 and 15 GHz was $\alpha = -0.67 \pm_{0.04}^{0.08}$ ($S_\nu \propto \nu^\alpha$, where S_ν is the flux density

TABLE 1 Description of antennas

Antennas	Diameter (m)	Aperture efficiency (K Jy^{-1})	System noise temperature (K)	Affiliation and location
B	100	1.75	60	Max-Planck-Institut für Radioastronomie, Effelsberg, Germany
D	70	0.72	21	NASA-JPL, Goldstone, CA, USA
G	43	0.254	45	NRAO, Green Bank, WV, USA
K	36	0.153	120	Haystack Observatory, Westford, MA, USA
M	70	0.60	30	NASA-JPL, Robledo, Spain
O	40	0.256	200	Owens Valley Radio Observatory, Big Pine, CA, USA
T	20	0.055	85	Onsala Space Observatory, Onsala, Sweden
Y	125*	1.89†	30	NRAO, 50 miles west of Socorro, NM, USA

* Effective diameter for the array of 25 antennas of 25 m diameter.

† Effective aperture efficiency for the phased array.

at frequency ν) (ref. 8) and not $\alpha > -0.3$, as expected for pulsar-powered plerions. However, we cannot rule out a composite morphology similar to that seen in some galactic supernova remnants: a shell with a weaker pulsar nebula in its centre. Further VLBI images of the supernova as it expands might reveal such a nebula as a central component distinguished from the shell.

The indication of a shell structure and the steep radio spectrum are consistent with a model in which a shock front of debris interacts with the circumstellar medium left over from the pre-supernova phase^{17,18}. In this model, the radio luminosity of the supernova depends on the circumstellar density and on the optical depth due to free-free absorption in the ambient medium outside the expanding shock front. As the supernova was optically thin at the time and frequency of the VLBI observations, the variations of the brightness along the rim could be explained in this model in terms of a directional dependence of the circumstellar density.

Protrusions are not expected in the simplest versions of any of the models discussed above, but could be caused by a number

of processes. Candidate processes include the expulsion of jets along the rotation axis of a collapsing core²⁰; Rayleigh-Taylor instabilities leading to a deformation of the shock front, as suggested²¹ for the supernova remnant 41.9+48 in the galaxy M82^{21,22,23}; the outbreak of plasma through weak points of the shell²⁴; and the formation of giant loops of magnetic field lines bulging out into the circumstellar medium¹⁶. The supernova will probably remain a sufficiently strong emitter of radio waves for further successful VLBI observations to be made for a few more years. As SN1986J is relatively young, a few years of monitoring will cover a large fraction of the supernova's lifetime and will therefore provide a much more complete record of the history of the evolution of a supernova, or its remnant, than we can reasonably expect through monitoring the expansion of supernova remnants in our own Galaxy. A sequence of images of SN1986J will not only help reveal the nature of the protrusions, but will also expose the evolution of each segment of the expanding shock front and show the interaction with the circumstellar medium, and finally, perhaps, give evidence for a pulsar concealed in the centre. $\square$

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A lattice-like construction to explain diffraction patterns of some non-stoichiometric phases

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ONE of the most significant advances in the understanding of non-stoichiometric compounds was Anderson's concept of infinitely adaptive structures¹. These materials exhibit a continuum of perfectly ordered structures as the stoichiometry is varied. X-ray crystallography has not yielded satisfactory structures for these phases, and high-resolution electron microscopy has also been unable to unravel the difficulties. To try to resolve these problems, we have focused our attention on the nature of the diffraction patterns that the compounds produce. We find that in many cases the diffraction patterns can be reproduced by the use of lattice-like constructions which we term shift lattices. Here we describe the method of construction of a one-dimensional shift lattice and mathematical representations of it and its Fourier transform. The method has wide applicability to complex structures, of which we have investigated several examples: the low-temperature tantalum pentoxide (L-Ta₂O₅) family of structures, Bi₂TeO₅-related phases in the Bi₂O₃-TeO₂ system and a number of non-stoichiometric heavy-metal oxyfluoride phases. For simplicity, we limit ourselves to a consideration of the one-dimensional case which we illustrate by reference to L-Ta₂O₅; nevertheless, all the examples that we

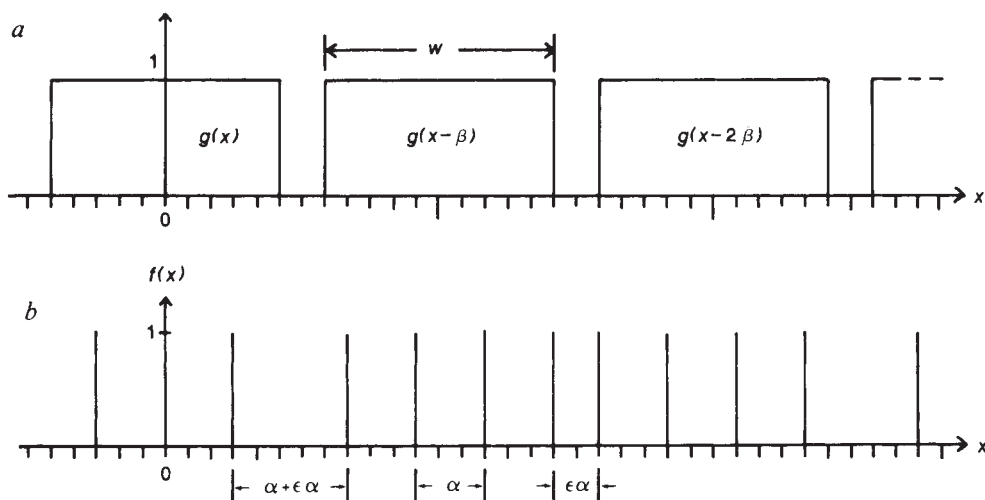
have considered are explicable by such one-dimensional shift lattices.

We assume that the one-dimensional structure, $f(x)$, where x is distance, consists of identical motifs, a motif at a position x_0 being represented by the delta function $\delta(x - x_0)$. A 'comb' of such delta functions, the 'teeth' of which are a distance α apart, is represented by $\delta_\alpha(x) = \sum_{n=-\infty}^{\infty} \delta(x - n\alpha)$. We then construct a periodic array of pulse functions, each of unit height and width w , a distance β apart, as depicted in Fig. 1a; these pulse functions, $g(x)$, $g(x - \beta)$ and so on, are not actually part of the structure but merely serve to define the limits of component parts of it. The structure itself is the sum of the products of g functions with shifted versions of $\delta_\alpha(x)$. For example, $g(x)$ itself is multiplied by $\delta_\alpha(x)$; $g(x - \beta)$ is multiplied by $\delta_\alpha(x - \epsilon\alpha)$, which is the same comb shifted by a distance $\epsilon\alpha$, where $0 < \epsilon < 1$. In general, the m th g function, $g(x - m\beta)$, is multiplied by $\delta_\alpha(x - m\epsilon\alpha)$, and so the whole structure, $f(x)$, is

$$f(x) = \sum_{m=-\infty}^{\infty} g(x - m\beta) \delta_\alpha(x - m\epsilon\alpha) \quad (1)$$

An example is shown in Fig. 1b for the case where $g(x)$ is an even function of x , $\alpha = 3$, $\beta = 12$, $\epsilon = 2/3$ and the width, w , of each g function is 10. The structure $f(x)$ resembles a lattice with quasi-regular shifts, these shifts being occasioned by the fractional shift, $\epsilon\alpha$, between the set of delta functions enveloped by one g function and that enveloped by its neighbour. We call an array of this type a shift lattice and the entity on the right-hand side of equation (1) a shift comb. For the parameters used in Fig. 1b, the shift lattice has a period of $3\beta = 36$, but for arbitrary β/α and ϵ , the period may vary enormously and may, indeed, be infinite. In all cases, however, there is long-range order.

FIG. 1 *a*, Periodic array of pulse functions $g(x)$, $g(x-\beta)$, $g(x-2\beta)$ and so on, each of width w . *b*, Part of the shift lattice $f(x)$. Each vertical line represents a delta function. The left-hand section of the structure is the product of $g(x)$ and a comb of spacing α . To its right is the product of $g(x-\beta)$ and an identical comb displaced by $\epsilon\alpha$. In general, the m th pulse function, $g(x-m\beta)$, is multiplied by a comb displaced by $m\epsilon\alpha$.



It is well-known that diffraction patterns are related essentially to their corresponding structures through Fourier transformation. The transform of $f(x)$, $F(u)$, where u is the reciprocal-space variable is

$$F(u) = \alpha^* \beta^* \sum_{h=-\infty}^{\infty} G(u - h\alpha^*) \delta_{\beta^*}(u - h\epsilon\beta^*) \quad (2)$$

where

$$E + \epsilon = \alpha^* \beta \quad (3)$$

$G(u)$ is the transform of $g(x)$, h is an integer, $\alpha^* = 1/\alpha$ and $\beta^* = 1/\beta$. The transform of a shift comb is therefore another shift comb (although the 'teeth' of the latter comb are not all of equal height). A schematic diagram of $F(u)$ is shown in Fig. 2. The envelopes G are identical, neighbouring envelopes being displaced by α^* ; neighbouring delta functions within one envelope are β^* apart, and there is a shift of $\epsilon\beta^*$ between combs enveloped by neighbouring G functions (as equation (2) shows). The fractional shift, ϵ , in real space may be estimated from measurement of α^* , β^* and $\epsilon\beta^*$, and the use of equation (3). This calculation normally yields a value for ϵ greater than unity; we are usually interested only in the fractional part because $\delta_{\alpha}(x) = \delta_{\alpha}(x - m\alpha)$ for any constant integer m . We would therefore reduce a result of, for example, $\epsilon = 4.66$ to $\epsilon = 0.66$.

We illustrate the real-space/reciprocal-space relationship for shift lattices by an optical example². Figure 3 shows a portion of a shift lattice which has been used in a study of the structures of the $\text{L-Ta}_2\text{O}_5$ family of compounds. Although at first sight this lattice seems to be two-dimensional, it has shift properties that are, in projection, one-dimensional only, and has been constructed with the parameters $\beta/\alpha = 8.0$, $w = 4.0$ and $\epsilon = 1/3$. The black dots in Fig. 3 correspond to transparent apertures in an

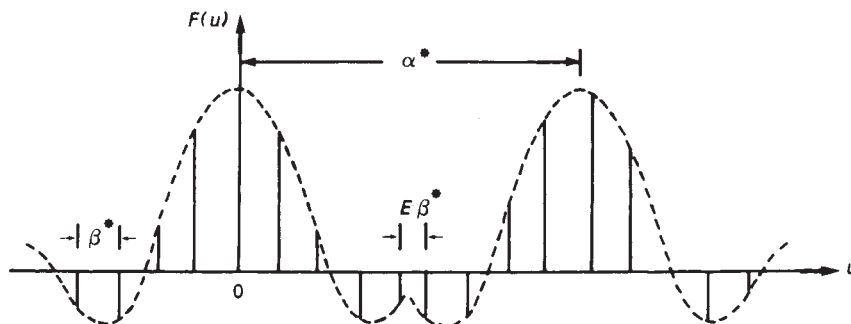
opaque diffracting screen which represent structural units in the material. At this stage it is not necessary to specify the precise form of these units, which might, for example, be cation-centred polyhedra or clusters of such polyhedra. The agreement between Fig. 4a, which is the optical transform of the mask shown in Fig. 3, and Fig. 4b, an electron diffraction pattern of $\text{L-Ta}_2\text{O}_5$, confirms that shift lattices give diffraction patterns that precisely mimic the features found in the experimental patterns.

The shift lattice is assembled by a mechanical procedure in which only the positions of neighbouring lattice points are relevant after values of α , β , ϵ and w have been selected. The construction can produce only completely ordered structures even though these may have infinitely long unit cells. This agrees with the observation that the systems examined have never shown disorder in either commensurate or incommensurate diffraction patterns.

In chemical terms, α is equivalent to a few metal-metal distances in the crystal, and $\epsilon\alpha$, which defines the position of the shift lattice with respect to the underlying matrix, is typically a cation-anion distance. Both of these parameters are thus controlled by normal chemical bonding. The large unit cells found can therefore be generated by short-range interactions, without recourse to long-range forces extending over hundreds of nanometres. The spacing, β^* , of the 'superlattice' spots in any one cluster in a diffraction pattern is not the inverse of the unit-cell dimension; if a cell of finite length exists, its size may be deduced from the parameters α , β and ϵ . High-resolution electron micrographs, which invariably image only small regions of crystal, do not allow, in our experience, any reasonable estimation of these parameters.

Stoichiometric or other variation over the phase range is introduced by altering the width, w , and/or separation, β , of the pulse functions. Reference to Fig. 3 shows that the shift lattice can be viewed as a coherent intergrowth of two different

FIG. 2 Schematic diagram of a portion of the Fourier transform $F(u)$ of a shift lattice, $f(x)$. The transform is similar in form to the structure itself, with shifts of the underlying lattice between envelopes. The envelopes are sinc functions; for the $\text{L-Ta}_2\text{O}_5$ structures, they have no significant overlap.



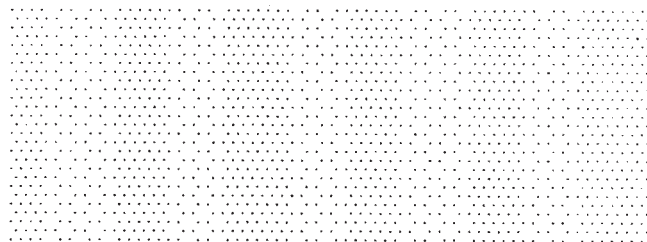


FIG. 3 Diffracting mask showing the shift-lattice model of tantalum pentoxide, which can be seen as the intergrowth of two different structures. The underlying lattice is hexagonal, vacancies occurring according to shift-lattice rules; the vacancies represent motifs of scattering power (in this case, zero) different from those represented by the dots. Only a portion of the mask is shown here.

portions. In the $L\text{-Ta}_2\text{O}_5$ family of phases, the two parts of the structure are considered to have different chemical compositions, and it is the continuous variation of their proportions, specified by the smooth variation of β , that accounts for the stoichiometry range of the family. The differences between parts of the structure need not be compositional. In magnetic oxides, for instance, the two regions may have different 'spin up' and 'spin down' configurations. Such a disparity would not be detected by X-rays or electrons, but could give rise to observable neutron diffraction effects. Semiconductor superlattices may be an example of another system which could be better characterized in terms of a shift lattice, although if the shift parameter ϵ is an integer the shift lattice reduces to a trivial construction.

Here we have outlined the principles of the one-dimensional shift lattice. It is also possible, by extending the concept to two dimensions, to reproduce diffraction features of the

type described in ref. 3 as "orientation and spacing anomalies". Fuller details of such applications as well as the three-dimensional generalization of the shift lattice will be published elsewhere. □

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Passivity breakdown and pitting corrosion of binary alloys

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PITTING corrosion—the localized dissolution of a passivated (oxide-covered) metal in the presence of a solution of certain anionic species—is a major cause of failure of metal structures. The breakdown of extremely thin (~1 nm thick), highly stable passivating layers typically occurs in a sporadic, localized and stochastic fashion^{1,2}, rather than as a catastrophic, global process. Using a model of a random binary iron–chromium alloy, we have shown previously^{3–6} that experimental observations of passivity of stainless steels can be explained by assuming that it is controlled by the selective dissolution of iron⁷. Thus if the chromium content is above a certain threshold (the percolation limit⁸), clusters of iron are finite and dissolution will proceed for a while and then stop. Oxidation of surface chromium atoms to form Cr–O–Cr linkages then creates a passive state in which the entire surface is covered with such a layer^{3,5}. Here we show that, by adding to this model the further assumption that there is a small but finite dissolution rate for surface chromium atoms, one obtains a mechanism for the triggering of pitting corrosion of stainless steels that is consistent with experimental studies.

The propagation of corrosion pits is generally thought to occur as follows. The corrosion current density within a developing pit is very large. The local environment is acidified owing to hydrolysis of the dissolving metal ions, and anions such as chloride are concentrated into the pit from the external solution because they carry the current flow. This establishes gradients in solution composition and electrode potential, which maintain the metal surface inside the pit in an active (dissolving) state, in contrast to the passive state outside. There is no agreement, however, on how pitting is initiated in the first place. On metals such as iron, nickel and aluminium, the current density in the passive state is of the order of a few $\mu\text{A cm}^{-2}$ (ref. 9), and in these cases simple feedback models can be developed^{10,11} in which the local passive dissolution current induces local fluctuations in solution composition, leading to a local acidification which causes further local increases in the passive dissolution rate. Such a local fluctuation might thus become amplified sufficiently to lead to pit initiation. The passive-current density on stainless steels, however, is very low (a few nA cm^{-2} after a few hours)^{11,12}, and the local chemistry required to initiate and sustain pitting corrosion is extreme (much more so than that for Fe, Ni or Al, for example): about 80% saturation in dissolved

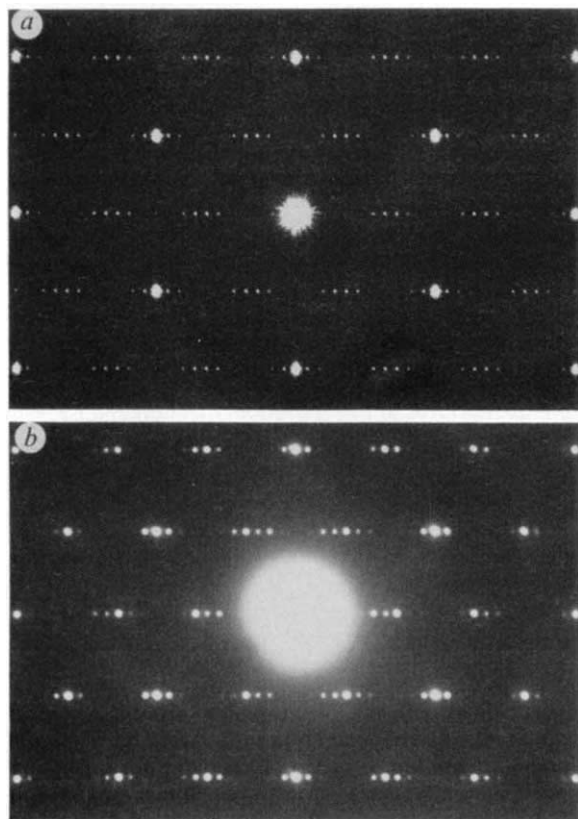


FIG. 4 *a*, Optical transform of the mask depicted in Fig. 3. *b*, Electron diffraction pattern of tantalum pentoxide $L\text{-Ta}_2\text{O}_5$.

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metal, $\text{pH} \approx 0$, $[\text{Cl}^-] > 6 \text{ M}$ (refs 13, 15). Simple feedback models, although not inconsistent with much experimental evidence^{1,11}, are therefore hard to sustain. There are several other hypotheses to explain breakdown^{13,17}, usually involving particular special assumptions: for example, accumulation of vacancy condensates at the metal–film interface¹⁸, the generation of critical-sized clusters of Cl^- at special sites¹⁹ or the presence of specific ‘weak spots’ in the passive film²⁰. Such special assumptions have also been incorporated into ideas of local

instability^{21–23}. Experimental evidence to support these models is only very indirect, and it is difficult to see how many of them can be distinguished experimentally.

Here we explore the consequences for pit nucleation of a simple modification of a percolation model for passivity developed previously^{3–6}, using a two-dimensional simulation of an iron–chromium alloy as a guide (Fig. 1). In this simulation, ‘passivity’ arises when the one-dimensional surface is completely covered by chromium atoms. In Fig. 1, we illustrate the

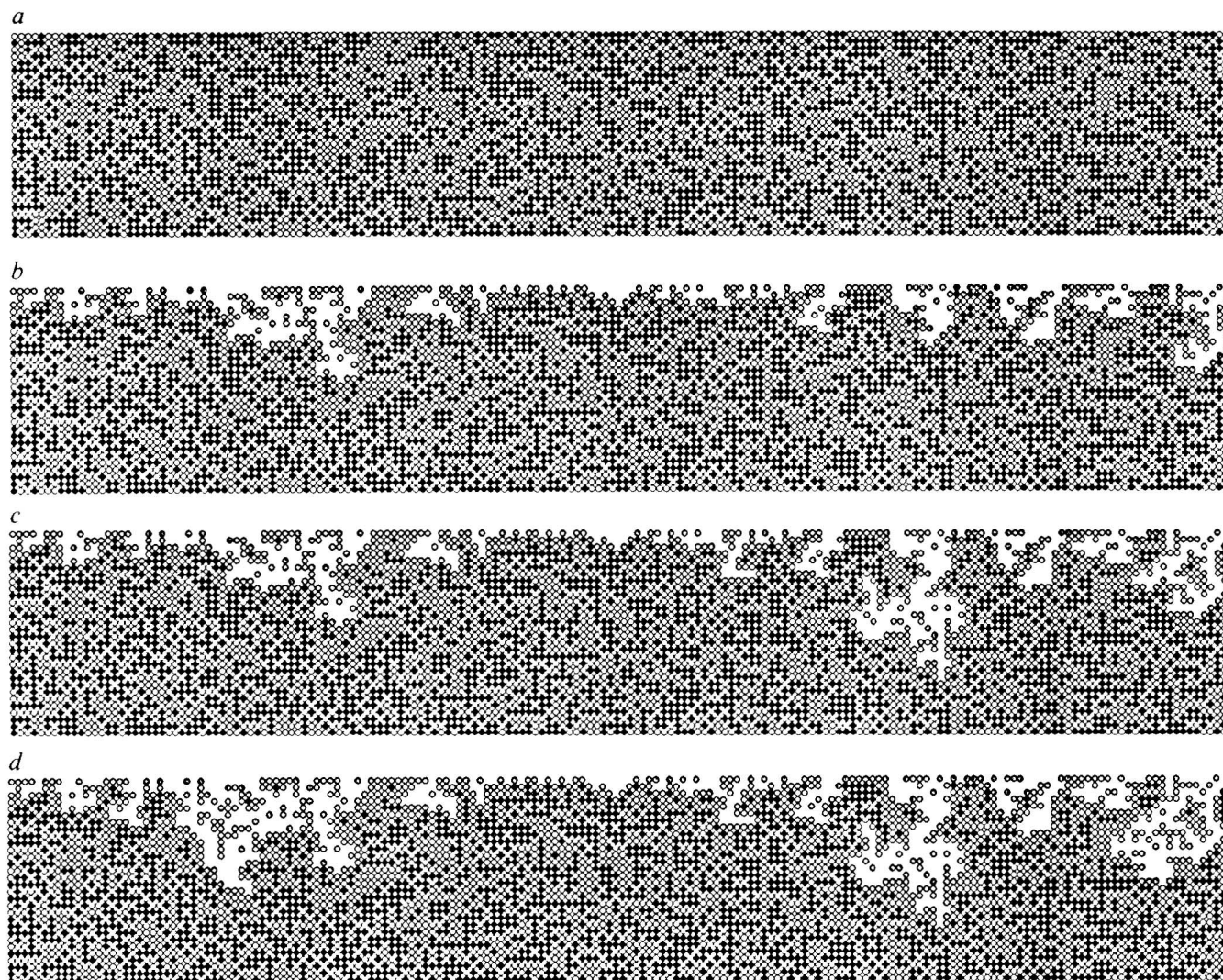
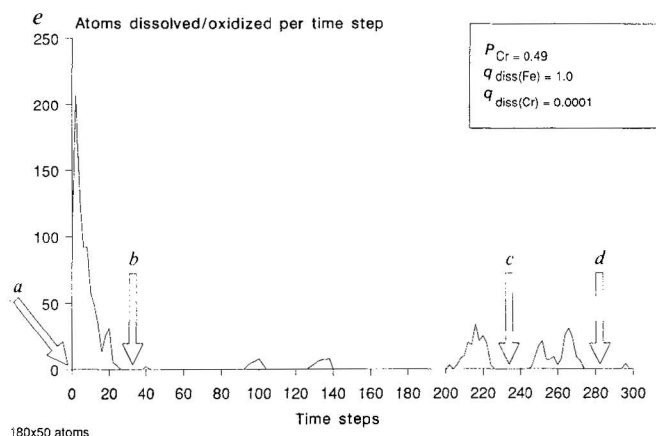


FIG. 1 Results of a two-dimensional simulation of dissolution of a binary alloy on a square lattice with a random distribution of Fe (●) and Cr (○) atoms, illustrating passivity when the concentration of Cr is above the percolation limit (40.7% Cr), above which point a connected path of Fe atoms cannot exist through the lattice. The chromium–atom fraction $P_{\text{Cr}} = 0.49$; dissolution probabilities, q_{diss} , are unity for Fe and 1×10^{-4} for Cr. The top face represents the solution interface. *a*, Initial state, illustrating Fe clusters both intersecting the surface and just below the surface. *b*, Configuration after initial passivation (following the removal of the iron clusters that intersected the original surface), showing the ramified interface that results. *c*, *d*, Configuration after dissolution of further clusters of Fe, following dissolution of a small number of atoms of Cr. *e*, Current transients for these simulations.



consequences of allowing a small dissolution probability of Cr. A random time series of current pulses appears (Fig. 1e) following the initial establishment of passivity. The frequency of occurrence of these pulses increases with increasing Cr dissolution probability, and the mean pulse amplitude decreases with increasing Cr content of the alloy above the percolation limit. The explanation for these pulses is simple. In any random binary lattice, there exist connected clusters of each component⁸. Above the percolation threshold for chromium, clusters of iron are finite in extent. Progressive dissolution of Cr atoms then uncovers iron clusters which subsequently dissolve, causing the current pulses; the charge distribution of the pulses thus reflects the cluster size distribution. In particular, very large clusters are very rare. Iron dissolution results in convoluted local penetrations of the lattice (Fig. 1).

To account for the triggering of pitting corrosion, we now propose simply that oxidation of the large number of surface Cr atoms within the convoluted cluster region would acidify the local volume (Fig. 2) and so could lead, if the cluster is large enough, to the establishment of the critical chemistry necessary for activation of the alloy. This idea provides a rational justification for the pit triggering processes proposed by several authors, whereby a critical local solution composition must be maintained for a critical time²⁴. It provides an explanation as to why 'feedback'¹¹ could develop locally, despite the very low average dissolution rate. The salient points are that pit nucleation is expected to be a rare event, because of the rarity of large iron clusters; that the pit generation rate should be proportional to the passive-current density (as observed¹¹), because this current density can be taken as a measure of the chromium dissolution probability; and that, as is well known, the pit generation rate decreases with increasing Cr concentration (above the percolation limit). Furthermore, we predict that very-low-level current transients with the appropriate frequency and size distribution should appear in suitably aggressive media that do not cause macroscopic pitting corrosion, such as acidic sulphate solutions or chloride solutions containing a large excess of an inert anion. We have recently observed such transients³¹.

This idea presented provides an appealing explanation for the triggering of pitting corrosion on stainless steels, but there are certain points of detail that need to be addressed. We have developed the idea using two-dimensional simulations. In the three-dimensional case, for body-centred cubic alloys, the percolation model leads to a passivity threshold composition that is consistent with observations only if iron atoms in terrace sites (five neighbours) are considered inert^{5,6}: the threshold otherwise corresponds to a very high Cr concentration. The very low passive-current density may be explained by assuming³ either that the terrace iron atoms are extremely unreactive or that the small spacing of the chains of Cr atoms that cover the ramified three-dimensional ledge network on the surface hinders the dissolution of Fe from the terrace sites, which then occurs only in a slow and selective fashion. The 'constructive dissolution' proposed by Burstein and Marshall⁷ could be a phase of establishment of passivity in which a surface morphology develops such that the dissolution of Fe from terrace sites is adequately blocked. Confining dissolution to ledge or kink sites renders most of the connected Fe clusters unavailable for dissolution.

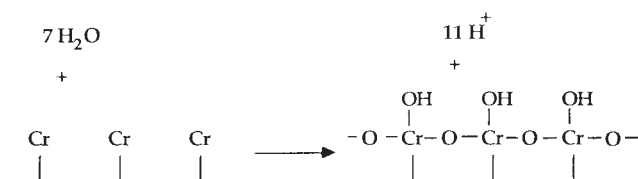


FIG. 2 Schematic illustration of acidification of a local volume as a result of oxidation of surface chromium atoms.

Indeed, in a three-dimensional simulation, the assumption of a small dissolution probability for Fe on terrace sites has much the same effect as the assumption of a small dissolution probability for Cr: current pulses occur following the initial establishment of passivity. There follows the further, interesting possibility³¹ that the developing acidic environment within the dissolving cluster volume increases the probability of dissolution of Fe from terrace sites, hence dramatically increasing the available cluster volume and increasing the likelihood that a pit may develop.

Transport of ions and water may be problematic within a region that has convolutions and interstices of atomic dimensions. But Fig. 1 implies that the dissolution process would leave Cr atoms on the surface in low-coordination states: surface diffusion may then act to coarsen the microstructure and consequently facilitate access into the cluster volume. The importance of surface diffusion processes has been recognized in earlier simulations of activation of passive metals⁶ and dealloying²⁵. A second, interesting possibility arises when we compare the development of the passive surface revealed by simulations such as that in Fig. 1 with conventional ideas concerning the thickening of passive layers. The latter, which are well developed and substantiated for layer growth on pure metals, propose that passive films thicken by ionic migration through the film, driven by a high electric field within the film. It is generally assumed (although with little direct evidence) that the very much thinner films formed on stainless steels (thickness ~ 1 nm or so²⁶) thicken in an analogous way. Our simulations, however, raise a different possibility for these alloys: specifically, that the apparent film thickening is, in part at least, due to field-driven migration of ions into and out of the convolutions that develop at the interface, and that the passive film whose properties are measured by volume-averaging techniques is in fact the highly convoluted interface region. The film should therefore appear to such techniques as a non-stoichiometric oxide, perhaps a bilayer structure or a structure having an apparent stoichiometry dependent on depth within the layer and exposure time, having some short-range order but no long-range order, incorporating water and perhaps anions non-uniformly over the surface to an extent also dependent on the depth within the layer and the time of exposure, and with an apparent thickness that increases with exposure time. This is exactly the interpretation given, with different emphases by different authors¹³, of extended X-ray absorption fine structure (EXAFS)²⁷, Auger electron spectroscopy (AES) and X-ray photoelectron spectroscopic results. It is stated frequently in interpretations of surface analytical measurements on the passive oxide on stainless steel that the layer contains hydrated material²⁸⁻³⁰, especially on steels with lower Cr content²⁹. According to our simulation, the lower the Cr content, the more convoluted is the interface.

As to why not all of the penetrations develop into pits, we suggest that pit development is determined by the size and geometry of the penetration. The probability of finding a very large cluster is very low, decaying exponentially with cluster size^{8,25}, and this, we suggest, accounts for the rarity with which penetrations develop into pits. According to our model, therefore, the following factors would be important in determining the probability of initiation of pitting corrosion on stainless steel: (1) the dissolution probability of chromium, which determines the rate of uncovering of the iron clusters (this would be dependent upon the local environment: it may be increased by species such as thiosulphate present in the vicinity of sulphide inclusions, which are preferred sites for pit nucleation on austenitic stainless steels¹²); (2) the alloy composition, which determines the cluster size distribution; and (3) diffusion and electromigration within the cluster volume as the metal dissolves, which determine the rate of accumulation of anions into the cluster, the rate of dissipation of the internal cluster environment to the external solution and the access of water into the cluster volume. □

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Revised projection of future greenhouse warming

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FOR the Intergovernmental Panel on Climate Change (IPCC) report¹, using a simple climate/ocean model, we made projections of the greenhouse warming to 2100. Projections were made for four greenhouse-gas scenarios, whose radiative effects in 2100, expressed in terms of an equivalent amount of CO₂, ranged from 2 to 5.5 times the pre-industrial CO₂ concentration. The projected global warming in 2100 for these scenarios, relative to 1990, ranged from 0.62–2.31 °C for the minimum assumed CO₂-doubling temperature sensitivity, $\Delta T_{2x} = 1.5$ °C, to 1.61–5.15 °C for the maximum sensitivity $\Delta T_{2x} = 4.5$ °C. Here we broaden these projections to include a recently suggested lower sensitivity, $\Delta T_{2x} = 0.5$ °C. We also revise all projections by prescribing, using the results of our analysis of simulations by a coupled atmosphere-ocean general circulation model, a lower value for a key parameter of the simple ocean model, Π , which indicates the warming of the polar ocean relative to the warming of the non-polar ocean. We find that, for any value of ΔT_{2x} , the atmospheric temperature increases more rapidly with time as a consequence of the reduction in Π . We also find that a delay of ten years in initiating a 20-year transition from the IPCC 'business-as-usual' scenario to any other IPCC scenario has only a small effect on the projected warming in 2100, regardless of the value of ΔT_{2x} . This indicates that the penalty for a 10-year delay is small.

Our earlier analysis for the IPCC report, and this revision thereof, both use an energy-balance climate/upwelling-diffusion ocean model (Fig. 1) similar to that introduced by Hoffert *et al.*² and used by Hoffert and Flannery³ to predict CO₂-induced climate change. This simple climate/ocean model

is used instead of our atmosphere-ocean general circulation model⁴ because of the latter's large computational requirement.

The simple climate/ocean model (Fig. 1) determines the surface temperature of the atmosphere and the temperature of the ocean as a function of depth from the ocean surface to the ocean floor. In the model, the ocean is subdivided vertically into 40 layers, with the uppermost being the mixed layer and the deeper layers each being 100 m thick. The ocean is also subdivided horizontally into a polar region where bottom water is formed, and a non-polar region where there is vertical upwelling. In the non-polar region, heat is transported upwards toward the surface by the upwelling, and downwards by physical processes whose effects are treated as equivalent to diffusion. Heat is also removed from the mixed layer in the non-polar region by transport to the polar region and downwelling towards the ocean bottom—this heat is ultimately transported upwards from the ocean floor in the non-polar region. Five quantities must be prescribed in this simple climate/ocean model: the temperature sensitivity of the climate system, characterized by the equilibrium warming induced by a CO₂ doubling, ΔT_{2x} ; the vertically uniform upwelling velocity for the global ocean, W ; the vertically uniform thermal diffusivity, κ , by which all non-advective vertical heat transport in the ocean is parameterized; the depth of the oceanic mixed layer, h ; and the warming of the polar ocean relative to the warming of the non-polar ocean, Π . For the IPCC report we chose three values of ΔT_{2x} (4.5, 2.5 and 1.5 °C) and $h = 70$ m, $W = 4$ m yr⁻¹, $\kappa = 0.63$ cm² s⁻¹, and $\Pi = 1.0$. We chose the latter value as a result of simulations by atmospheric GCM/mixed-layer ocean models of the equilibrium climate change induced by a doubling of the atmosphere CO₂ concentration⁵. These simulations show a poleward amplification of the surface temperature change in the winter hemisphere, thereby suggesting that Π should equal 1.0.

Here we prescribe Π based on our recent analysis⁴ of the transient time evolutions of $1 \times \text{CO}_2$ and $2 \times \text{CO}_2$ simulations with an atmosphere-ocean GCM. Examining the changes in temperature in the polar (downwelling) and non-polar (upwelling) regions gives values of Π that range from 0.569 for the uppermost layer (a depth of 0–50 m) to 0.004 for the lowermost layer (a depth of 2,750–4,350 m), with a depth-averaged value of 0.161. The smallness of the depth-averaged value and the value of Π for the deep ocean are probably due to the brevity of the $1 \times \text{CO}_2$ and $2 \times \text{CO}_2$ simulations, each being only 20 years

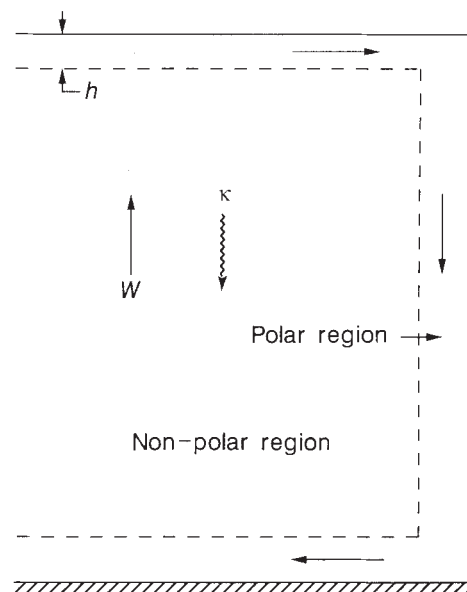


FIG. 1 Schematic representation of the energy-balance climate/upwelling-diffusion ocean model.

TABLE 1 Potential warming reduction in 2100 obtained by a linear transition from IPCC scenario A to IPCC scenario B, C or D during either 1990–2010 or 2000–2020

ΔT_{2x} (°C)	Scenario A to B		Scenario A to C		Scenario A to D	
	1990–2010 (°C)	2000–2020 (°C)	1990–2010 (°C)	2000–2020 (°C)	1990–2010 (°C)	2000–2020 (°C)
0.5	0.39	0.37 (96%)	0.55	0.52 (96%)	0.64	0.61 (96%)
1.5	1.04	0.99 (95%)	1.45	1.38 (96%)	1.71	1.63 (95%)
4.5	2.31	2.18 (95%)	3.17	3.00 (95%)	3.78	3.57 (95%)

long. It is therefore likely that $0.161 < \Pi < 0.569$, with a value closer to the mean of 0.365, and we accordingly choose $\Pi = 0.4$.

We have calculated the temperature change from 1765 to 1990 induced by the historical evolution of greenhouse gases (T. Wigley, personal communication), and from 1990 to 2100 for the four IPCC scenarios. These scenarios can be characterized by the year in which the radiative effect of greenhouse gases is equivalent to a doubling of the pre-industrial CO_2 concentration: A ('business-as-usual'), 2015; B, 2031; C, 2038 and D, 2100. We have performed these calculations for presumed temperature sensitivities of $\Delta T_{2x} = 4.5$, 1.5 and 0.5 °C, the first two values being the extremes adopted by IPCC, and the latter value proposed by Lindzen⁶ (and personal communication, 1990). Comparing the calculated greenhouse-induced temperature changes for these different sensitivities with the observed global mean temperature changes from 1880 to 1990 (ref. 1) shows that the patterns of temperature change are not identical in shape or magnitude (Fig. 2). In particular, the calculated temperature changes increase monotonically in time and attain values of 1.30, 0.61 and 0.24 °C in 1990 relative to 1880 for the three sensitivities, respectively, but the observed global mean temperature does not increase monotonically in time, and is ~ 0.5 °C in 1990 relative to 1880. Thus, there are significant disparities between the observed and reconstructed temperature evolutions for both $\Delta T_{2x} = 4.5$ and 0.5 °C, with a best fit being obtained for $\Delta T_{2x} = 1.24$ °C, a value less than the minimum temperature sensitivity used in the IPCC calculations. This sensitivity is also below that for zero feedback (only radiative-convective adjustment of temperature without changes in other quantities such as water vapour, clouds and sea ice), $(\Delta T_{2x})_0 = 1.32$ °C, and implies a net negative feedback of $f = -0.06$ [$\Delta T_{2x}/(\Delta T_{2x})_0 = 1/(1-f)$]. But as discussed by Wigley and Raper⁷, it is difficult to estimate ΔT_{2x} from the observed temperature record because of the unknown contribution from the climate's natural variability.

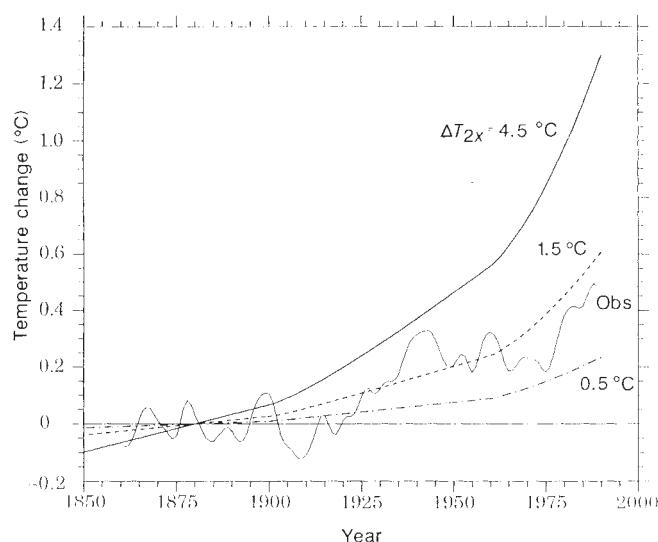


FIG. 2 Historical greenhouse-gas-induced temperature change calculated from 1765 to 1990 for prescribed climate sensitivities of $\Delta T_{2x} = 4.5$, 1.5, and 0.5 °C, together with the observed temperature change (Obs). All temperature changes have been referenced to 1880.

Any estimate of ΔT_{2x} is further confounded by external forcing mechanisms, both natural (for example, volcanic and solar-irradiance variations) and anthropogenic (for example, sulphate emissions⁸), the magnitudes of which are also uncertain.

Our temperature projections to 2100 for the IPCC scenarios using $\Pi = 0.4$ give larger temperature increases than those using $\Pi = 1.0$, with the difference being larger for larger ΔT_{2x} . This occurs because less heat is transferred to the deep ocean as Π decreases, hence the warming of the upper ocean and atmosphere increases. For the business-as-usual scenario (A) and with $\Delta T_{2x} = 4.5$ °C, $T(2100) - T(1880) = 6.2$ °C for $\Pi = 1.0$, but 7.2 °C for $\Pi = 0.4$. The corresponding values for $\Delta T_{2x} = 1.5$ °C are 2.9 °C and 3.1 °C, whereas those for $\Delta T_{2x} = 0.5$ °C are both 1.1 °C. These results clearly show that the magnitude of the potential greenhouse-gas-induced climate change ranges from catastrophic to minor depending on the true value of ΔT_{2x} for the climate system. Consequently, it is imperative to narrow the range of possible values of ΔT_{2x} , for example, by determining the minimum possible value below which the Earth would not have undergone glacial-interglacial cycles. This can be done by model simulations of these cycles and comparison with paloclimate data.

We have calculated the temperature changes from 1990 to 2100 for the IPCC scenarios B, C and D, in which greenhouse gas emissions are reduced. These calculations show, as did our IPCC calculations, that reducing the future increase in greenhouse gases reduces the future increase in temperature, with the size of the reduction being larger for larger ΔT_{2x} . For example, the 1990–2100 temperature rise for scenario B is less than that for scenario A by 2.45, 1.08 and 0.40 °C for $\Delta T_{2x} = 4.5$, 1.5 and 0.5 °C, respectively. If a linear transition from 1990 to 2010 is

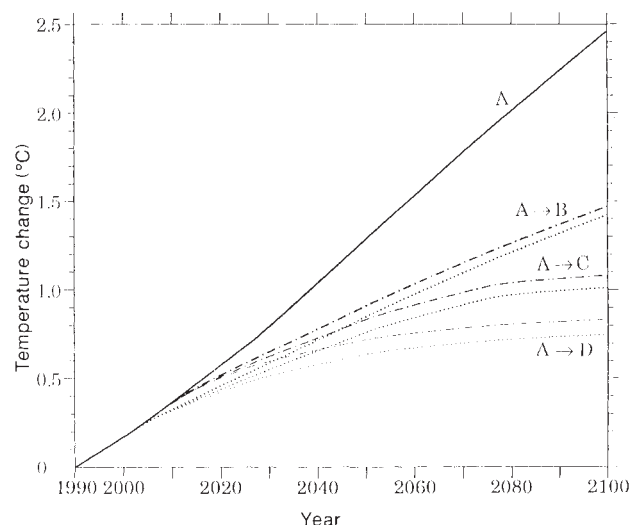


FIG. 3 Projected greenhouse-gas-induced temperature change to 2100 for a prescribed climate sensitivity of $\Delta T_{2x} = 1.5$ for: IPCC scenario A (solid line); a linear transition from 1990 to 2010 from IPCC scenario A to either IPCC scenario B, C or D (dotted lines); and a linear transition during 2000 to 2020 from IPCC scenario A to either IPCC scenario B, C or D (dash-dotted lines). All calculations begin in 1765 and all temperature changes are relative to 1990.

made from the emission rate of scenario A to the emission rate of scenario B, the reduction in temperature rise in 2100 is 2.31, 1.04 and 0.39 °C for $\Delta T_{2x} = 4.5$, 1.5 and 0.5 °C, respectively (Fig. 3, Table 1). If the initiation of this transition in emission rate is deferred 10 years until 2000, the reduction in temperature rise in 2100 is 2.18, 0.99 and 0.37 °C; that is, at least 95% of that possible by not deferring the transition. The equivalent atmosphere CO₂ concentration in 2100 increases as a consequence of this deferral, thereby increasing the equilibrium temperature change (the 'committed warming,' approximately equal to the increase in temperature change shown in Fig. 3) by 0.15, 0.05 and 0.02 °C for $\Delta T_{2x} = 4.5$, 1.5 and 0.5 °C, respectively. Corresponding results are obtained for 20-year linear transitions from the emission rate of scenario A to the emission rate of either scenario C or scenario D (Fig. 3), with the reduction in temperature rise in 2100 equal to at least 95% of that possible by not deferring the transition, regardless of the temperature sensitivity (Table 1). The corresponding increases in the committed warming in 2100 for a 10-year deferral in transitions from scenario A to C(D) are 0.22(0.28), 0.07(0.09) and 0.02(0.03) °C for $\Delta T_{2x} = 4.5$, 1.5 and 0.5 °C, respectively. This indicates that the penalty is small for a 10-year delay in initiating the transition to a regime in which greenhouse-gas emissions are reduced.

To us this small penalty does not indicate that we should 'wait and see' and do nothing during this decade—quite the contrary. The study of the greenhouse effect, both theoretically and observationally, should be accelerated into a 'crash programme' so that we do not squander the time that nature has given us to obtain a realistic understanding of the climate response to increasing concentrations of greenhouse gases. □

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Impact of oceanic sources of biogenic sulphur on sulphate aerosol concentrations at Mawson, Antarctica

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SULPHATE is the dominant aerosol species in the Antarctic atmosphere^{1,2} and an important constituent in Antarctic snow and ice³. Various sources have been suggested for Antarctic non-sea-salt sulphate (n.s.s. SO₄²⁻): volcanic emissions, stratospheric injection, pollutants transported from the low latitudes and biogenic dimethylsulphide (DMS) from the ocean^{1,2}. Although the oceanic source is now believed to be especially important, there has been no strong chemical evidence directly linking oceanic DMS with the Antarctic n.s.s. SO₄²⁻ concentrations. Here we present extended measurements from the Antarctic for both n.s.s. SO₄²⁻ and

methanesulphonate (MSA), an oxidation product of DMS. Both species have a very strong seasonal cycle with a maximum in the austral summer; this cycle parallels that of the oceanic biogenic sulphur producers, thereby suggesting a strong link between the Antarctic atmospheric sulphur cycle and biological processes in the Southern Ocean.

The sulphate ion is the oxidative endmember of the atmospheric sulphur cycle and it is one of the principal species in aerosols and precipitation over large areas of the Earth. Globally, anthropogenic emissions of SO₂ are the largest source of atmospheric sulphur⁴ whereas the second largest source is believed to be oceanic emissions of reduced organic sulphur, particularly DMS^{5–7}. There is considerable interest in oceanic DMS emissions because of a possible link with climate. High concentrations of n.s.s. SO₄²⁻ aerosol could conceivably have a significant impact on the aerosol albedo⁸, on cloud nucleation processes and cloud lifetimes and on cloud optical properties⁹. The oceanic sources of sulphur are of particular interest because of the hypothesized feedback links between the biota and climate¹⁰. The temporal and spatial variability of oceanic DMS emissions over the southern oceans and the Antarctic is especially interesting because of the possibility that measurements of n.s.s. SO₄²⁻ in snow and ice cores could be used to characterize the past history of oceanic DMS sources and the possible link with palaeoclimate.

Aerosol samples were collected continuously at Mawson, Antarctica, (67° 36' S, 62° 53' E) between February 1987 and October 1989 with the assistance of the Australian National Antarctic Research Expeditions (ANARE) (Fig. 1). A high-volume filter sampler draws air continuously through a 20 × 25 cm² polypropylene mat-type filter (Delbag Microdon) for one-week periods; the collection efficiency of the filters is essentially 100% for submicrometre aerosols. Operational blank filters are periodically exposed. Because of the location of the sampler at Mawson and the highly directional (katabatic) wind regime, there is no evidence of significant contamination from local sources. Filters are stored and batches are periodically returned to the United States for analysis. A portion of the filter is extracted with water which is then analysed for NO₃⁻, SO₄²⁻, Cl⁻ and MSA (all by ion chromatography), for Na⁺ (by atomic absorption) and for NH₄⁺ (by colorimetry). A portion of each filter is also measured for the natural radionuclides ⁷Be and ²¹⁰Pb, but at this time we present the results of the analysis only for SO₄²⁻ and MSA. Our SO₄²⁻ data refer only to n.s.s. SO₄²⁻, that fraction of the SO₄²⁻ mass which is derived from sources other than dissolved salts in sea water. The sea-salt fraction (which is subtracted from the total SO₄²⁻) is calculated on the basis of the Na⁺ concentration using the SO₄²⁻/Na⁺ ratio in sea water, 0.2517. Annual means (calculated only on the basis of the first two complete years of data) are presented in Table 1 along with comparative data from other locations.

TABLE 1 Mean concentrations of non-sea-salt sulphate and MSA measured in aerosols at Mawson (this work) and comparative data from several other locations

Constituent	Units	Mawson*	Neumayer†	South Pole‡	Cape Grim§	American Samoa
N.s.s. sulphate	ng m ⁻³	90	(71)¶	83	280	360
MSA	ng m ⁻³	20	—	—	18	26

* This work. Annual means are computed for the period 20 February 1987 to 20 February 1989.

† Wagenbach *et al.* 1988 (ref. 20).

‡ Tuncel *et al.* 1989 (ref. 21).

§ Ayers *et al.* 1986 (ref. 23).

|| Savoie *et al.* 1989 (ref. 7).

¶ This value is calculated by us from data presented by Wagenbach *et al.*²⁰. It is based on an annual mean total sulphate concentration (164 ng m⁻³) calculated from the means given for November to March (278 ng m⁻³) and April to October (82 ng m⁻³); we calculated the n.s.s. SO₄²⁻ concentration from the mean sodium concentration (369 ng m⁻³) which we estimated from the reported mean sea-salt concentration of 1.2 µg m⁻³.

The weekly n.s.s. SO_4^{2-} concentrations over the 32-month period show a seasonal pattern that is striking in both its amplitude and its consistency (Fig. 2). The monthly mean n.s.s. SO_4^{2-} concentrations during the summer are 30–40 times higher than those during the winter (210 and 250 ng m^{-3} during December and January as opposed to 8.2 and 6.5 ng m^{-3} during June and July). The relatively low scatter of the winter data for n.s.s. SO_4^{2-} and MSA is taken as evidence that filter blank concentrations and contamination levels are very low and relatively constant and that the variability due to analytical procedures is small.

Our MSA data (Fig. 2) are unique for the Antarctic. These data show a very strong annual cycle that peaks in the austral summer. The highest monthly mean concentrations, typically in the range 30–60 ng m^{-3} , occur from December to March. During the winter, the concentrations are ~10–30 times lower with means of 2–3 ng m^{-3} from June to August.

The seasonality of our MSA cycle agrees with the seasonal cycle of DMS concentrations in coastal waters off Davis Station, Antarctica (68°35'S, 77°52'E)¹¹. Concentrations were extremely low during the winter (0.2–0.5 nmol l^{-1}), but they began to increase sharply in November, reaching a maximum in December (190 nmol l^{-1} at a depth of 7 m). DMS concentrations were highly correlated with cell counts of *Phaeocystis pouchetii*, a unicellular alga which has been associated with DMS emissions in other ocean regions.

The annual MSA cycle at Mawson is very similar to that for n.s.s. SO_4^{2-} (Fig. 2). But whereas n.s.s. SO_4^{2-} peaks in December–January, MSA reaches a maximum a little later and subsequently drops very rapidly. Accordingly, the ratio MSA to n.s.s. SO_4^{2-} ranges from ~0.15 at the beginning of the summer to nearly 0.4 at the end. The mean ratio, 0.22, is much higher than that measured in the low-to-middle latitudes of the South Pacific where mean values are typically ~0.06–0.07 (refs 12–14). This suggests that the atmospheric chemistry controlling the oxidation of DMS (primarily by reaction with OH radicals) to MSA and SO_2 may be quite different in the Antarctic, possibly because of temperature effects¹⁵.

The atmospheric circulation patterns for the Antarctic support the conclusion that the concentrations measured at Mawson are not necessarily limited to the coastal region, but rather that they could be representative of a relatively large area of the interior of the continent^{1,2}. The single most dominant meteorological feature over Antarctica is the katabatic flow (gravity-forced drainage) of radiatively cooled air from the elevated interior

plateau, down the marginal ice slopes, to the coast¹⁶. At Mawson, winds flow from a narrow inland sector more than 80% of the time¹⁷. The katabatic flow prevents a strong transport of sea salt directly to Mawson by onshore winds, even during the winter, and results in low sea-salt concentrations (Table 1). These factors suggest that ocean air reaching Mawson must first have travelled a considerable distance inland.

Clearly, the persistent surface-level outflow from Antarctica must be balanced by inflow at higher altitudes. Results from simple models^{18,19} indicate that the low-level outflow induces a cyclonic vortex in the upper troposphere. James¹⁹ concluded that the polar vortex is most probably disrupted by strong interactions with decaying mid-latitude depression systems which spiral toward the pole. This mid-to-upper tropospheric inflow is believed to be responsible for the transport of most of the aerosol particles and/or their precursors to the Antarctic continent¹.

The concentrations of n.s.s. SO_4^{2-} and MSA (and also sea salt) at Mawson do not appear to be strongly affected by the proximity to the open ocean as determined by sea-ice coverage. Satellite imagery shows that ice coverage is at a maximum in October when it extends out to ~60°S, a distance of ~700 km from Mawson; ice is at a minimum in February at which time there is little or no sea ice in the Mawson region (W. Betts,

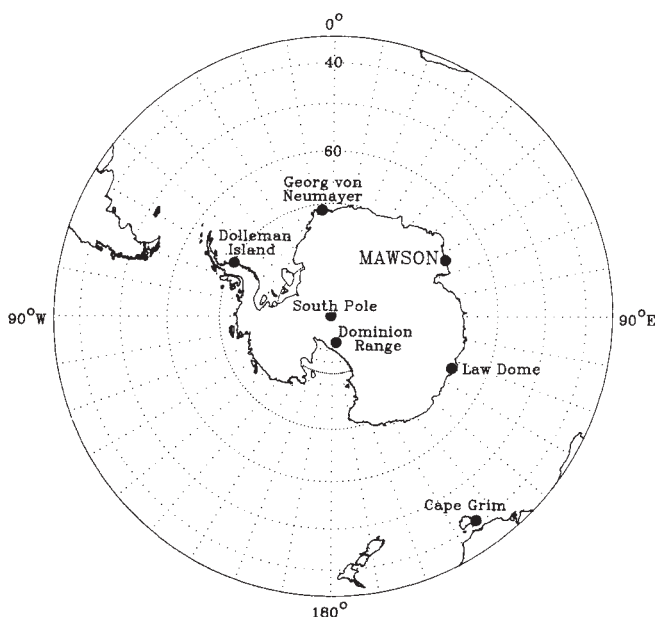


FIG. 1 Map of the Antarctic continent.

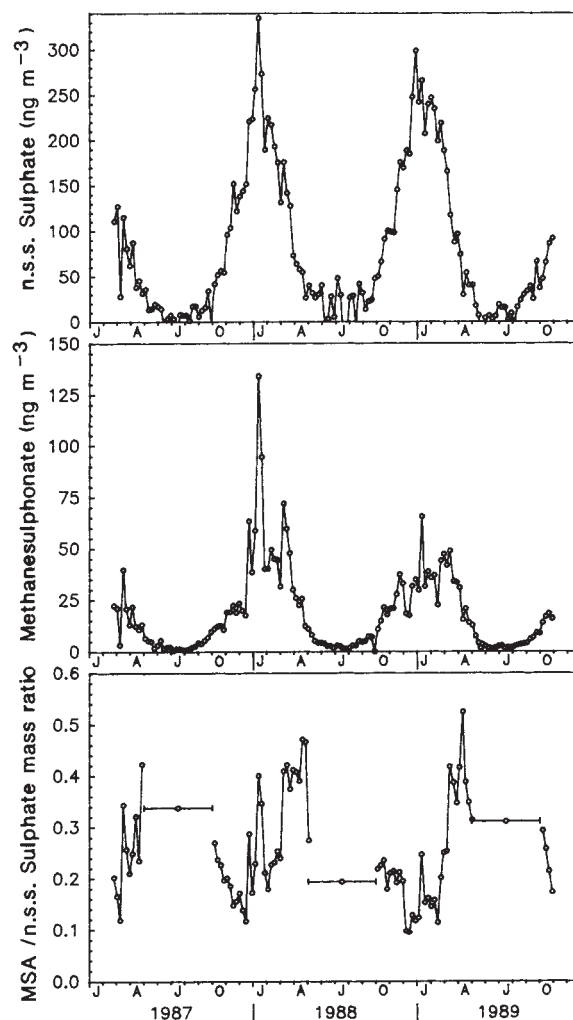


FIG. 2 The concentration of non-sea-salt sulphate (top) and MSA (centre) in weekly aerosol samples collected at Mawson, Antarctica, February 1987 to October 1989. (Units: 10^{-9} g m^{-3} of air, STP). The weight ratio of MSA to n.s.s. SO_4^{2-} is shown at the bottom; for the winter, when the n.s.s. SO_4^{2-} and MSA concentrations are extremely low and the ratios are highly variable, we present the mean values for May–September.

personal communication). Thus, n.s.s. SO_4^{2-} and MSA concentrations (Fig. 2) begin to increase sharply near the time of ice maximum and they attain their maximum (or near-maximum) values well before ice minimum.

The seasonal n.s.s. SO_4^{2-} cycle at Mawson is quite similar to that observed at Georg von Neumayer (GvN) station ($70^\circ 37' \text{S}$, $08^\circ 22' \text{W}$)²⁰. The peak (summer) n.s.s. SO_4^{2-} values are also similar. We cannot easily compare our winter data with GvN where they report long periods when n.s.s. SO_4^{2-} values are negative (because of high sea-salt concentrations). Nonetheless, we can conclude that the GvN n.s.s. SO_4^{2-} concentrations are extremely low during the winter. The similarity in n.s.s. SO_4^{2-} values and seasonal cycles at Mawson and GvN is rather remarkable considering that these stations are separated by over 3,000 km. This similarity implies that the concentrations are controlled by large-scale processes related to both the distribution of sulphur sources and the atmospheric mixing and transport patterns. The only other extensive n.s.s. SO_4^{2-} data for the Antarctic are for the South Pole²¹ where the annual mean is quite similar to that found at Mawson and GvN (Table 1).

Our data are relevant to measurements of MSA and n.s.s. SO_4^{2-} in Antarctic snow and ice cores. These have been used to reconstruct an historical record of the atmospheric sulphur cycle. At Law Dome²² ($66^\circ 30' \text{S}$, 113°E), located relatively close to the coast, n.s.s. SO_4^{2-} exhibited a summer maximum and the mean MSA/n.s.s. SO_4^{2-} ratio was ~ 0.4 , which is comparable to the ratio in Mawson aerosol in the later summer. We have recently analysed an ice core from Dolleman Island in the Weddell Sea (Fig. 1) in collaboration with the British Antarctic Survey. These data also yield a mean MSA/n.s.s. SO_4^{2-} ratio of 0.4, with a strong summertime n.s.s. SO_4^{2-} maximum. There are few data from inland regions, but in a core from the Dominion Range (Fig. 1), the ratios are lower, ~ 0.05 (E. Saltzman, unpublished data) suggesting that the oceanic source may not be as important in the high central regions of the continent or that the sulphur is derived from mid-latitude oceanic sources where the MSA/n.s.s. SO_4^{2-} ratio is low¹²⁻¹⁴.

The aerosol data and the ice-core data, coupled with our knowledge of the meteorology of the Antarctic, suggest that the biogenic sulphur from oceanic sources plays a primary role in the atmospheric sulphur cycle of a substantial portion of the Antarctic and possibly over a very large area of the Southern Ocean. A multi-year MSA record from Cape Grim, Tasmania²³ ($40^\circ 41' \text{S}$, $144^\circ 41' \text{W}$) yields annual mean concentrations which are essentially identical to those at Mawson (Table 1). In recent studies at Cape Grim²⁴ the concentrations of MSA and n.s.s. SO_4^{2-} were measured concurrently with DMS over a two year period, 1988-89; all three species are closely correlated. The concentrations and seasonality of MSA and n.s.s. SO_4^{2-} were quite similar to those measured by us over the same time period at Mawson.

The similarity in the seasonal cycles and concentrations of MSA and n.s.s. SO_4^{2-} at Mawson and Cape Grim suggests that a large latitudinal range (at least 30°) of the Southern Ocean is dominated by these sources. This conclusion is supported by the n.s.s. SO_4^{2-} data from GvN (which is located almost 180° of longitude away from Cape Grim—see Fig. 1). Hence changes in the emission rates of biogenic sulphur from Antarctic waters should have a readily detectable, and relatively uniform, effect on the atmospheric chemistry of the region. Thus the concentration of these species in snow and ice (especially on the periphery of the Antarctic continent) should provide an accurate record of past oceanic emissions for the southern oceans as a whole.

For the oceans in general, it has been shown that increased DMS emissions are broadly associated with regions of increased primary productivity and that emissions follow a similar annual cycle^{6,7}. Hence, changes in productivity could affect the atmospheric sulphur cycle. Recent studies by Martin *et al.*²⁵ in the Antarctic indicate that the low productivity in these nutrient-rich waters might be due to iron deficiency. It has therefore been

suggested that the addition of Fe could enhance primary productivity by as much as an order of magnitude, thereby removing an amount of atmospheric CO_2 equivalent to the annual anthropogenic increment⁶. If the emissions of DMS were to increase proportionately, then the biogenic source could yield monthly mean summer n.s.s. SO_4^{2-} concentrations of $\sim 2 \mu\text{g m}^{-3}$, a value comparable to those measured off the east coast of the United States²⁷. Thus, if the hypothesized linkages of n.s.s. SO_4^{2-} to climate are valid^{8,10}, it is conceivable that changes in oceanic productivity, both natural and man-made, could affect climate over an immense region of the Southern Ocean and possibly over a large area of the Antarctic continent. $\square$

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Chemical removal of nitrate from water

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HIGH levels of nitrate in ground water can pose a serious health risk. Reduction of nitrate to nitrite in the gut may cause methemoglobinemia¹ both in newborn infants and in adults deficient in glucose-phosphate dehydrogenase². Under abnormal circumstances, reduction to nitrite can also occur in the stomach to form N-nitrosamines, a postulated cause of stomach cancer³. Nitrate outflow onto shallow continental shelves can promote nearshore algal blooms. Both natural and anthropogenic sources contribute to nitrate pollution. In the United States⁴ and Europe⁵, legislation now specifies a maximum permissible nitrate level in drinking water. Techniques such as selective ion exchange⁶, reverse osmosis, electrodialysis and distillation exist to transfer nitrate between two bodies of water, but only biological processes are presently available for nitrate destruction. Here I describe a chemical process in which aluminium powder reduces nitrate to ammonia, nitrogen and nitrite. In a pH range of 9 to 10.5, selective reduction of nitrate relative to sulphate is possible, and between pH 9.1 and 9.3, loss of the reductant through decomposition of water can be minimized to less than 2%. Subsequent control of pH and

concentrations of dissolved aluminium, nitrite and ammonia should be possible at a realistic cost, making this process potentially useful for combating nitrate pollution.

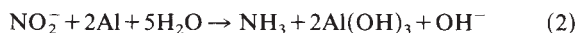
Powdered aluminium, a powerful reductant that can decompose water, sulphate and nitrate⁷, has an optimum pH (Fig. 1a) for nitrate reduction relative to sulphate. No reduction of either nitrate or sulphate (Fig. 1b) occurs at pH 8 because of the protective oxide coating on the aluminium particles. The rate of dissolution of the alumina on the metal particles increases with pH. When the pH is too high (Fig. 1c), aluminium is also consumed in sulphate reduction. As a crude comparison with these results, note that maximum legal nitrate levels in drinking water are 44 mg NO₃⁻ l⁻¹ in the United States⁴ and 50 mg NO₃⁻ l⁻¹ in Europe⁵ (effective from 1988).

The removal of the nitrate ion with powdered aluminium may first occur by adsorption onto the particles. Soon afterwards, however, products from the reduction of nitrate can be measured. Figure 2 shows a nitrogen mass balance. These tests, run at ambient temperature under slightly different operating conditions (such as pH, ratio of Al to NO₃⁻, initial nitrate concentration, total reaction time), show ammonia to be the principal reaction product (60–95%) followed by nitrogen gas and nitrite.

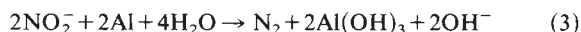
The reaction probably proceeds to nitrite



and from nitrite to either ammonia



or nitrogen gas



and it may be possible to optimize conditions for nitrogen gas production. Aluminium can be consumed in water decomposition

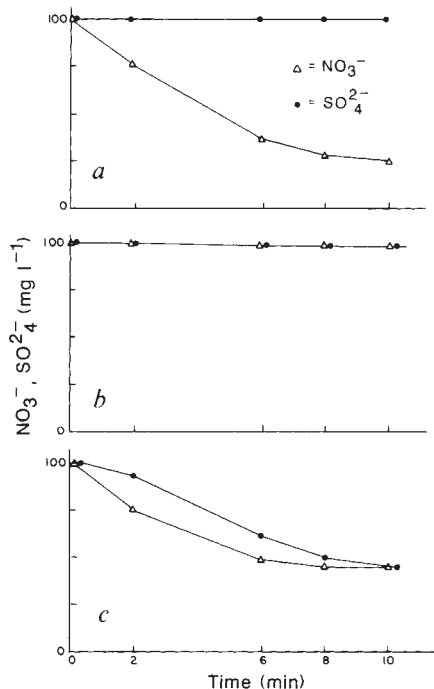
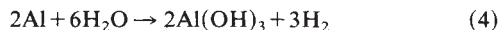


FIG. 1 Effect of aluminium on nitrate removal. a, pH 10.25; b, pH 8.00; c, pH 11.5. Conditions: room temperature; 450 ml of 100 mg l⁻¹ chloride, nitrate and sulphate with 1-g sample of 350-mesh aluminium powder. The pH was adjusted and maintained with an autotitrator. Samples were filtered through a 0.45-μm membrane and analysed by ion chromatography. Chloride concentrations did not change.

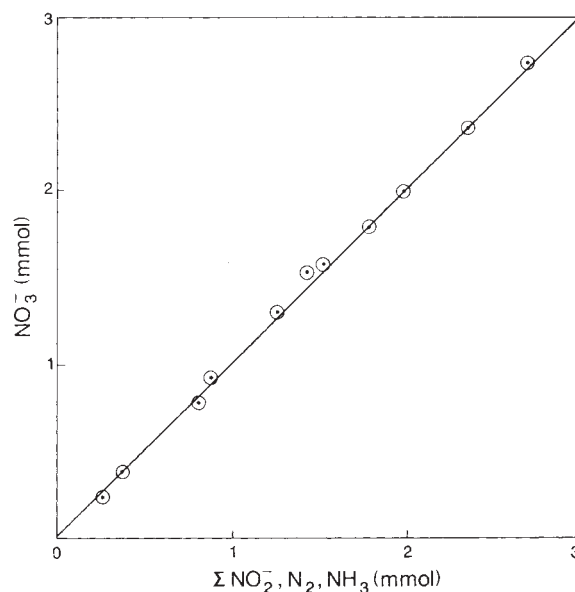


FIG. 2 Nitrogen mass balance. Conditions: room temperature; sealed glass reactor with argon purge operated at various conditions of pH, mass of aluminium powder, nitrate concentrations and reaction times. Nitrogen was determined by gas chromatography and ammonia was determined colorimetrically. Nitrate and nitrite were determined by ion chromatography.

Figure 3 shows that if the process is run in a narrower pH range, loss of aluminium by this reaction can be minimized to less than 2%.

Although this process is preliminary⁸, aluminium powder for nitrate control could be included in existing water treatment facilities. Many treatment plants use lime for water softening and removal of heavy metals⁹, raising the pH above 9.1 and then adjusting the unbuffered water down to pH 6–7 at the end. Such plants would require little additional chemical cost for the pH change necessary for the aluminium process.

This process may require air stripping or other techniques¹⁰ to remove the ammonia from water. Treatment facilities using air stripping would need little extra capital equipment costs but these costs would be incurred for other applications. The process could be flexible and aid in the disinfection process by providing

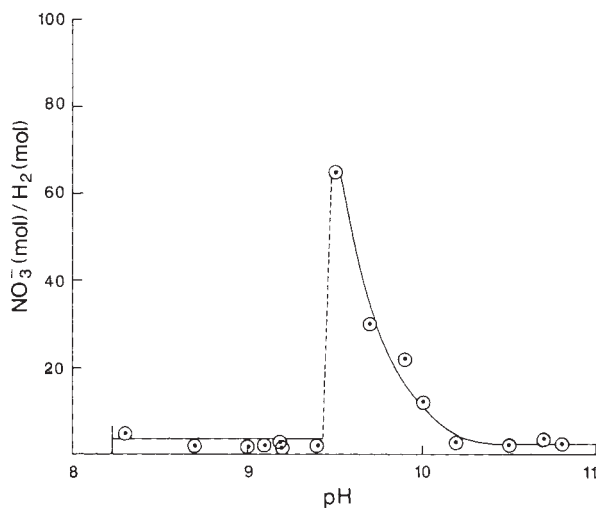


FIG. 3 pH range maximizing the ratio of nitrate reduction to hydrogen formation. Conditions: room temperature; sealed glass reactor with pH as the only variable. Hydrogen was determined by gas chromatography and nitrate by ion chromatography.

ammonia which in combination with chlorine forms chloramines. Chloramines can prolong the stability of residual disinfectant during distribution and can eliminate certain organic byproducts resulting from chlorination¹¹. Residual amounts of ammonia and nitrite may be neutralized by final chlorination. Ammonia is oxidized to nitrogen gas¹² and nitrite to nitrate¹³.

Chemical analyses on the final effluent from the process (pH adjusted to 6.2) demonstrate total aluminium to be $<0.03 \text{ mg l}^{-1}$. This is below the levels of aluminium in drinking water considered to be safe in the United States⁴.

If it is assumed that only ammonia is produced (maximum Al/NO_3^- required) and that the aluminium wasted in water decomposition can be ignored because hydrogen formed from reaction (4) represents $<2\%$ of the total aluminium used, then the three-electron change for aluminium oxidation ($\text{Al} \rightarrow \text{Al}^{3+} + 3\text{e}^-$) and the eight-electron change for nitrate to ammonia reduction mean that 1.16 g of aluminium are required to reduce 1 g of nitrate. □

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Dimensions and dynamics of co-ignimbrite eruption columns

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VERY powerful volcanic eruptions cannot always form classical Plinian eruption columns¹⁻⁴. Instead, collapsing fountains may develop above the vent and shed pyroclastic flows which spread laterally along the ground⁵. The upper part of these hot, dense pyroclastic flows may become buoyant through the entrainment, heating and expansion of ambient air, coupled with the sedimentation of larger clasts suspended in the flow. The buoyant material may rise, in a co-ignimbrite eruption column, carrying massive quantities of fine dust and volatiles into the stratosphere^{6,7}. Here we present a model of this process, and show that the co-ignimbrite columns associated with the eruptions of Toba^{8,9} 75,000 years ago and Tambora¹⁰ in 1815 may have ascended only about 32 and 23 km; the latter is comparable with the less powerful 1982 Plinian eruption column of El Chichón¹¹. This corroborates arguments that the mass of sulphuric acid aerosols injected into the stratosphere and not the eruptive power determines the climatic impact of an eruption^{12,13}.

The classical Plinian eruption column is formed when very hot, dense material is ejected upwards from the volcanic vent at high speed ($\sim 300 \text{ m s}^{-1}$)^{4,14-16}. This column entrains ambient air, which rapidly heats up and expands; the resulting buoyant plume may then rise tens of kilometres into the atmosphere (Fig. 1a). But if the mass eruption rate or vent radius is too large or

the eruption velocity too small, the material rising in the lower column will not become buoyant, and a collapsing fountain, only a few kilometres high, forms^{1-4,16,17} (Fig. 1b). Dense, hot pyroclastic flows may spread laterally from this fountain^{1,5}. As these develop, pyroclasts sediment out and entrained overlying air expands, lowering the density of the mixture. If sufficient air is entrained, material peels off the top of the current and may develop into a coherent, buoyant plume (Fig. 1c). In this way the thermal energy of a collapsing fountain may be transferred into a vast buoyant plume, and massive quantities of erupted material may penetrate high into the atmosphere. This phenomenon has been simulated in both analogue laboratory experiments²⁰ and numerical modelling¹⁵.

Co-ignimbrite columns developed during many of the most powerful historical eruptions²¹, including Tambora (1815)^{10,18}, Krakatau (1883)¹⁹ and Katmai (1912). In the Tambora eruption, the pyroclastic flows entered the sea $\sim 20 \text{ km}$ from the vent and the explosive interaction with evaporating surface water contributed to the development of the co-ignimbrite column^{10,18}. Co-ignimbrite columns are also believed to have formed in several immense pre-historic eruptions⁷ including the Aira caldera eruption in southern Kyushu, 21,000 y BP (before present)^{22,7}, the Minoan eruption in Santorini, 3,500 y BP^{8,23} and the Toba eruption in Sumatra, 75,000 y BP^{8,9}.

Here we extend the eruption column model of Woods¹⁴, using the conservation of mass, momentum and enthalpy and the entrainment assumption for a well mixed, buoyant plume²⁵ to simulate a steady-state co-ignimbrite eruption column. We assume the mixture of fine, hot ash and entrained air that rises

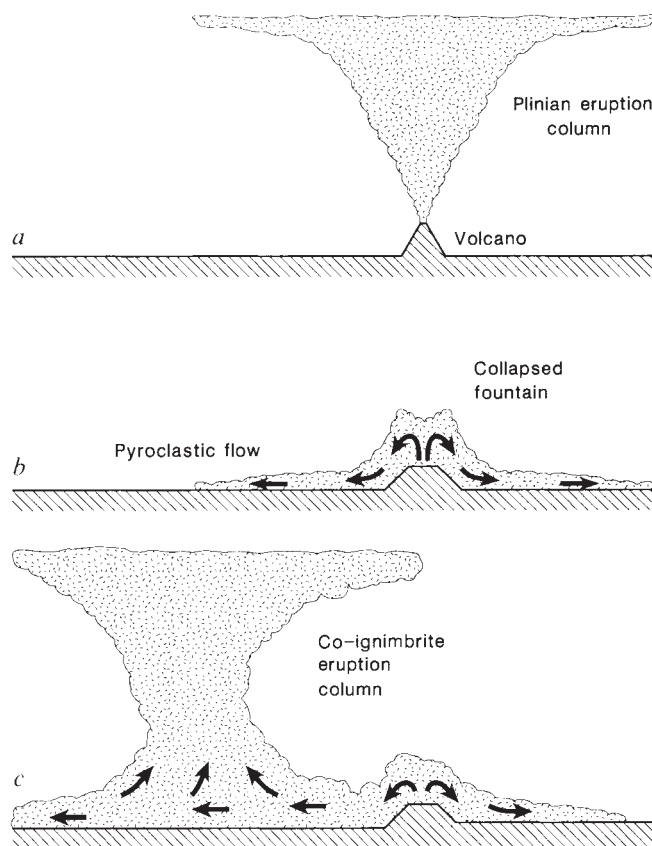


FIG. 1 Schematic of a, Plinian eruption column; b, fountain shedding lateral pyroclastic flows soon after the collapse of a Plinian column; c, development of a co-ignimbrite column formed as the buoyant elutriated mixture of fine ash and volatiles rises off the pyroclastic flow. The centre of the co-ignimbrite column may be displaced laterally from the vent²⁴ because of asymmetries in the pyroclastic flows, resulting perhaps from the topography of the volcano or the presence of a body of water.

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TABLE 1 Predicted and reported eruption column statistics

Eruption	Total volume erupted (km ³ dense rock equivalent)	Volume of ash elutriated† (km ³ dense rock equivalent)	Duration of co-ignimbrite column‡	Mass eruption rate from vent (10 ⁹ kg s ⁻¹)	Column height§ (km)	Mass of sulphuric acid aerosols (10 ¹⁰ kg)
Toba ^{8,9} 75,000 yr BP	2,840	840	9–14 days	7.1	32 ± 5*	—
Tambora ^{10,12,27} 1815	50	20	2–3 days	0.5	23 ± 3*	15–20
Mount St. Helens ²⁴ 18 May 1980 lateral blast¶	0.13	0.03	30–80 s*	7 ± 3*	25 ± 1	—
El Chichón ^{11,12} 1982 (Plinian)#	1.1	1.1	—	0.05	22 ± 4	1–2

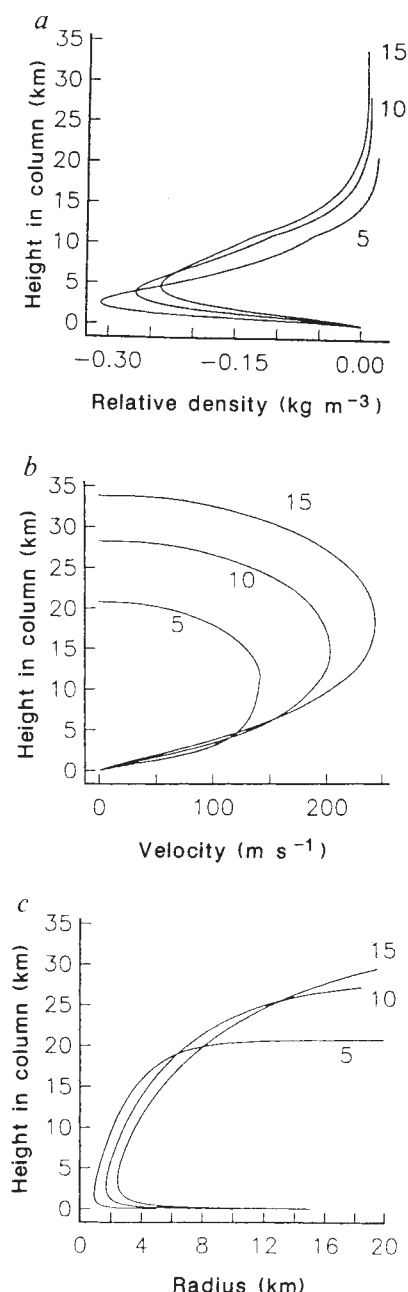
* Calculated herein.

† From the pyroclastic flows into the co-ignimbrite column.

‡ Estimated from deep-sea cores for Toba⁸ and historical evidence for Tambora^{10,27}.§ Toba and Tambora co-ignimbrite column heights are estimated from the model (error margins include uncertainties in initial conditions); the prediction for Toba is much lower than that of Ninkovich *et al.*⁸, consistent with the field data of Rose and Chesner⁹.

|| Injected into stratosphere.

¶ In the text we describe the ash cloud as an instantaneous co-ignimbrite thermal, rather than a maintained co-ignimbrite column; it had an observed ascent time of 6–8 minutes.

Average of the A, B and C eruptions¹¹.

off the pyroclastic flow to feed the column is well mixed, is in thermal equilibrium and is just buoyant relative to the ambient. The hot, elutriated material continues mixing with air, which expands, decreasing the bulk density of the mixture below that of the ambient (Fig. 2a). This buoyancy causes an upward acceleration of material (Fig. 2b), which initially causes the mass flux per unit area to increase with height faster than the rate of addition of mass through entrainment of ambient. The column radius therefore decreases to conserve mass (Fig. 2c) and the column approaches the natural shape of a buoyant plume.

Figure 3 shows how the total height of our model co-ignimbrite eruption column depends upon the total mass flux and temperature of the material erupted at the vent. Using field data, Sparks and Walker⁷ argued that a fraction, $\lambda > 0.35$, of the total erupted juvenile material will be elutriated from the pyroclastic flow and rise in the co-ignimbrite column. Recent data from Toba⁹ and Tambora¹⁰ also suggest that $\lambda \approx 0.35 \pm 0.05$ (Table 1), and we used this value for the typical calculations shown in Fig. 3. The development of the co-ignimbrite column may restrict the entrainment of air into the pyroclastic flows subsequently erupted, causing their areas to increase²⁶; however, the presence of water may limit the lateral extent of these flows, as occurred in Tambora¹⁰. An important result of our numerical investigations is that for a given mass eruption rate the total height of a co-ignimbrite eruption column is relatively insensitive to the initial elutriation velocity (0.1–10 m s⁻¹) or equivalently the area of the pyroclastic flows. Also, the column height increases with eruption temperature and, to a lesser extent, with the altitude of the tropopause so that co-ignimbrite columns nearer the equator tend to be higher.

Figure 3 shows for comparison the total height of a mid-latitude Plinian column with an eruption velocity of 300 m s⁻¹ and eruption temperature of 1,100 K. For a given erupted mass

FIG. 2 Calculations showing the variation with height in a co-ignimbrite eruption column of *a*, average density of material relative to ambient density at that height, *b*, average vertical velocity and *c*, radius, assuming axisymmetry. Numbers on the curves represent the radius (km) of the pyroclastic flow that supplies material to the co-ignimbrite column. The temperature of the material erupting at the vent is 1,000 K and the initial velocity of elutriation from the pyroclastic flow is 1 m s⁻¹; other values are as in Woods¹⁴. The mixture of fine ash and entrained air rising from the pyroclastic flow into the co-ignimbrite column is just buoyant and has cooled to 832 K. The very rapid acceleration in the lower part of the column (*b*) extends above the height at which the cloud has its minimum radius (*c*). As the hot, dusty material rises off the ground, it entrains air which heats up, expands and increases the buoyancy of the cloud (*a*). Higher in the column, the density difference decreases as the thermal energy available to generate buoyancy in the cloud is diminished.

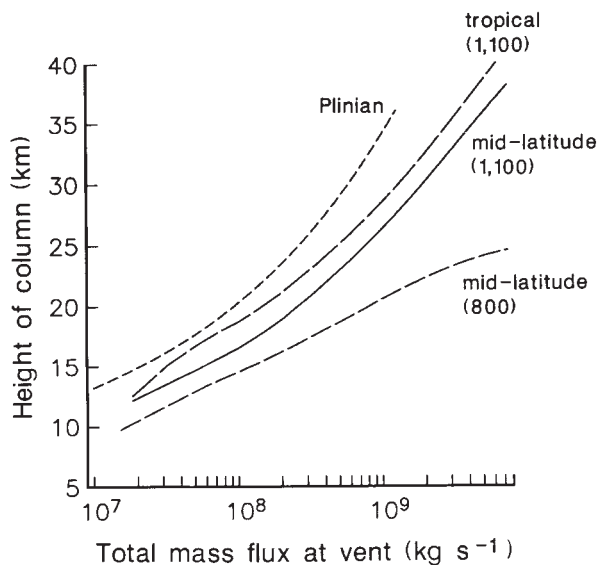


FIG. 3 A typical calculation of the total height of the co-ignimbrite eruption column as a function of the mass eruption rate at the vent. The ash cloud is assumed to have an initial velocity of 1 m s^{-1} . The numbers in brackets are two typical eruption temperatures⁵ (1,100 K and 800 K). The co-ignimbrite columns are assumed to convect 35% of the erupted material into the atmosphere^{7,9,10}. Other eruption properties are taken from lateral blast data²⁴ for Mount St. Helens. Two types of atmosphere, a mid-latitude atmosphere with the tropopause at 11 km and a tropical atmosphere with the tropopause at 15 km. Also shown is a Plinian column which is erupted into the mid-latitude environment with an initial velocity of 300 m s^{-1} and an initial temperature of 1,100 K.

flux, a Plinian column rises much higher than a co-ignimbrite column, primarily because nearly all the hot erupted pyroclasts convect upwards in a Plinian column (Fig. 1a). Furthermore, material which erupts to form a Plinian column contains a gas mass fraction of only 1–5% at the vent^{1,2}; large volumes of air are only entrained at higher altitudes, where the air is less dense. In contrast, material in the pyroclastic flow entrains a very large mass of the relatively dense, cool air just above the ground. This air is convected up into the co-ignimbrite column, lowering the temperature of the mixture a few hundred degrees below eruption temperature and contributing 30–60% of the total mass rising from the flow; for large mass eruption rates ($\geq 10^9 \text{ kg s}^{-1}$) or low eruption temperatures this further decreases the height of the co-ignimbrite column relative to that of the Plinian column. But in very powerful eruptions, Plinian columns only develop if the eruption velocity is very large; vent erosion rapidly lowers this velocity for a fixed mass eruption rate and a collapsing fountain forms^{1–4,17}. In this case, the only means of injecting material high into the atmosphere is a co-ignimbrite column; for example, over 92% of air-fall from the 1815 Tambora eruption was co-ignimbrite ash¹⁰.

Table 1 shows the predicted mass eruption rate at the vent that would have been required to maintain the 25-km cloud observed during the Mount St Helens lateral blast on 18 May 1980, assuming 23% (ref. 24) of the erupted material is entrained into the cloud; this calculation implies the column only lasted 1–2 minutes, whereas the reported ascent time of the cloud was 6–8 min²⁴. This cloud may be better described as a 'co-ignimbrite thermal' rising off the pyroclastic flow almost instantaneously²⁵ rather than continuously; such a blast cloud could ascend to 25 km (ref. 24). It may be that only the much larger eruptions, for example 1815 Tambora, can generate sufficient material over a sufficiently long time to maintain a co-ignimbrite column.

Table 1 also compares our estimates of the heights of two co-ignimbrite columns (Toba and 1815 Tambora) with the Plinian eruption column of El Chichón (1982). Although the 1815 Tambora eruption produced 50 times as much material as the El Chichón eruption and had 10 times the power, the column only reached a height similar to the El Chichón Plinian column, injecting 20 times as much ash into the stratosphere. This is a particularly important result, because it supports the argument¹² that eruptive power does not determine the climatological impact of a volcanic eruption; very powerful eruptions forming co-ignimbrite columns cannot inject material significantly higher than much weaker Plinian eruptions. Our low estimates for the injection height of co-ignimbrite ash also support arguments

that much of the volcanic ash falls out of the atmosphere too rapidly to have a long-term impact upon climate^{13,27}.

We reiterate¹² that it is the mass of sulphur gas injected into the stratosphere, where it forms sulphuric acid aerosols, which has the most important long-term effect upon climate. This mass is a function of the magma chemistry as well as of total mass erupted. Per unit mass of erupted material, Tambora injected one-fifth as much sulphur into the stratosphere as El Chichón. This is because El Chichón (1) was particularly sulphur rich¹² and (2) produced a Plinian rather than co-ignimbrite column.

Perhaps our most significant result is that the most powerful eruptions can inject massive quantities of ash 20–35 km into the atmosphere even if a Plinian column cannot develop. Simple estimates suggest that this ash, which mainly consists of small glassy shards^{6,7} with high drag coefficients⁹, is dispersed by atmospheric winds²⁷ to form a blanket of airfall several centimetres thick, over an area of 10^5 – 10^7 km^2 . This is consistent with field data^{7–10}. □

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Long-term memory of individual neighbours in a migratory songbird

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CAPABILITIES for long-term memory and recall of information have evolved in non-human animals primarily for special requirements such as for learning species-typical vocalizations and caching food¹⁻⁶. Long-term memory of individual social partners has, however, not been demonstrated previously for non-human animals. The ability to recognize individuals has important consequences for the evolution of intricate social interactions⁷⁻¹¹ and provides a basis for more sophisticated forms of cognition in animal societies^{12,13}. Recognition of social partners has been documented for territorial songbirds, which discriminate between songs of different neighbours¹⁴⁻¹⁶ as well as between the songs of strangers and neighbours¹⁷. Here I show that male hooded warblers (*Wilsonia citrina*, Parulinae) not only recognize their neighbours individually by song during the breeding season, but also retain the memory of neighbours' songs after an 8-month period during which they cease singing and migrate to Central America before they return to former breeding territories.

I studied 25 mated pairs of hooded warblers at Mason Farm Biological Reserve, Chapel Hill, North Carolina, from 1987 to 1989. Males in this population returned from migration in April and established territorial boundaries with neighbouring males. During territorial establishment, I tape-recorded each male on at least two separate occasions. I mapped all boundaries by noting locations of singing and encounters between neighbouring males.

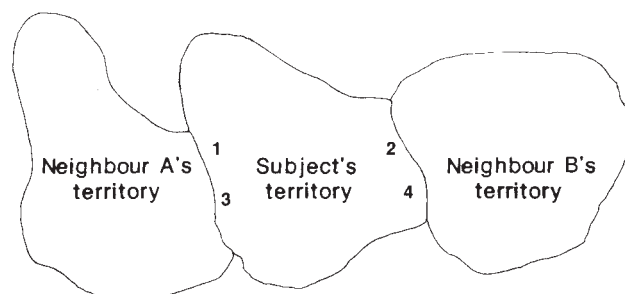
In 1988, playbacks of the tape-recordings demonstrated that males recognized each other individually by song. Hooded warblers in this population, like related species¹⁸, had repertoires of 2-9 song patterns used in two modes of singing. In the first mode, which predominated early in the breeding season, each male sang a single, individually distinctive pattern repeatedly (Fig. 1). For each male, I prepared a 3-min tape simulating the repeat mode of singing with six songs per minute. During May, I played tapes of two neighbouring males' songs to each of 12 male subjects from locations 15-20 m inside the subject's territory (Fig. 2). Each neighbour's song was presented near both correct and opposite boundaries of the subject's territory (N and XN playbacks, respectively).

To standardize the subject's behaviour and to ensure that he was within hearing distance, playbacks began only after the

subject had been singing for 3 min, 25 to 50 m from the speaker. Because the playback simulated a neighbour's singing, both the neighbour, whose song was used for playback, and the opposite neighbour, whose boundary was to be used for the XN test, had to be silent before playback could begin. For 3 min during the playback and for 9 min afterwards, I noted the subject's songs, flights, total time in the vicinity of, and closest approach to, the speaker.

Males approached more rapidly and spent more time near the speaker both during and after XN than N playbacks (Fig. 3). Males responding to XN playbacks often flew back and forth, as if looking for the intruder, and perched frequently on or near the speaker. Because these responses are probably not independent, I used principal component analysis to compute a composite score. One-way analysis of variance on these scores confirmed that males responded more strongly to XN playbacks (trial 1: $F_{1,22} = 18.028$, $P < 0.001$; trial 2: $F_{1,22} = 19.85$, $P < 0.001$). I conclude that males learned to associate each neighbour's song with its usual location, a form of individual recognition.

As in other migratory species¹⁹⁻²¹, returning male hooded warblers that had held adjacent territories in previous years engaged in fewer and less intense interactions than did males with new opponents. This reduction in interactions could result from mutual recognition of former territorial boundaries or of former neighbours. Recognition of neighbours' songs, however, would require memory of their characteristics for at least 8 months, as there is no evidence that neighbouring males either migrate together or sing in Central America²². Playback experiments in 1989 demonstrated that returning male hooded warblers



Day	Sample Playback		Protocol	
	Song presented	Location	Test	
1	A	1	N	
2	A	2	XN	
10	B	3	XN	
11	B	4	N	

FIG. 2 Schematic representation of the experimental protocol. The enclosed areas are territories of a subject and two neighbours, A and B. Each neighbour's song was presented twice, once near the boundary that the subject shared with that neighbour (neighbour test; N) and once near the boundary on the opposite side of the subject's territory (cross-neighbour; XN). For the first playback, location 1 was selected 15 to 20 m inside the subject's territory, the speaker was placed 2-2.5 m above the ground and connected with a 20-m lead to the monitor output of a Sony TC-D5M recorder. All playbacks were standardized at 90 dB 1 m away, a level typical for singing birds. A coin toss then determined which neighbour's song would be played, with the constraint that equal numbers of subjects received XN and N playbacks first. On the next day at the same time, the same tape was played back on the opposite side of the subject's territory at location 2. Twice playbacks attracted a neighbouring male who interacted with the subject. These playbacks were terminated and repeated 5 days later. For these tests, 12 different playback tapes were used for the 12 subjects. Seven to ten days later, similar tests with each subject used the songs of the other neighbour at locations 3 and 4, 5-10 m away from the previous locations in order to reduce the possibility of subjects' becoming sensitized to specific locations. The complete experiment included 16 different playback tapes; the total is less than 24 (12 subjects $\times$ 2 neighbours each) because some subjects shared neighbours.

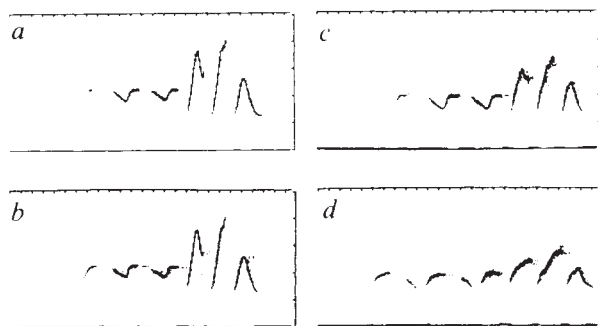


FIG. 1 Spectrograms (vertical divisions, 2 kHz; horizontal divisions, 61.5 ms) of song patterns used by three hooded warblers. *a, b*, Songs used in the repeat mode by the same male recorded on different days. *c, d*, Songs used in the repeat mode by two neighbouring males.

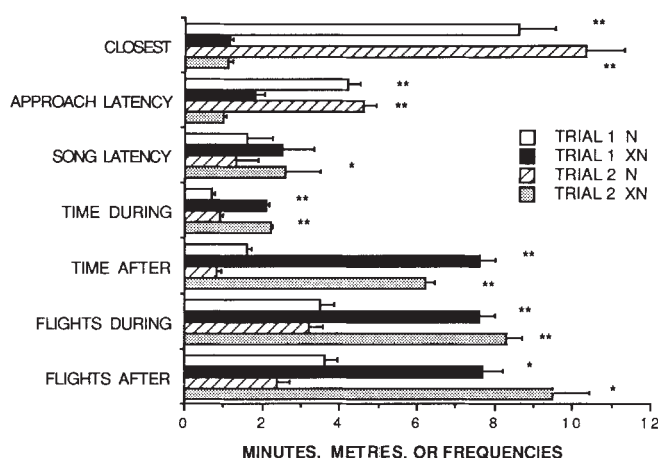


FIG. 3 Average responses and standard errors for male hooded warblers responding to playbacks of neighbours' songs on the correct (N) and opposite (XN) boundaries of the subjects' territories. Responses to current year's neighbours ($N = 12$), with the first series of N and XN tests (trial 1) separated from the second series (trial 2, 7–10 days later). Responses are the closest approach to the speaker (m/2), latency (min) to approach the speaker within 10 m, latency (min) to the first song (responding males usually approached the speaker quietly and only began singing after the playback ended, whereas males that did not respond usually continued singing in the same manner as before the playback), time (min) spent within 10 m of the speaker during and for 9 min after the playback, and the number of flights during and after the playback. A strong response is indicated by small values for closest approach and latency to approach and large values for the other five measures. **, $P < 0.01$, Wilcoxon matched-pairs test (1-tailed); *, $P < 0.05$, Wilcoxon matched-pairs test (1-tailed).

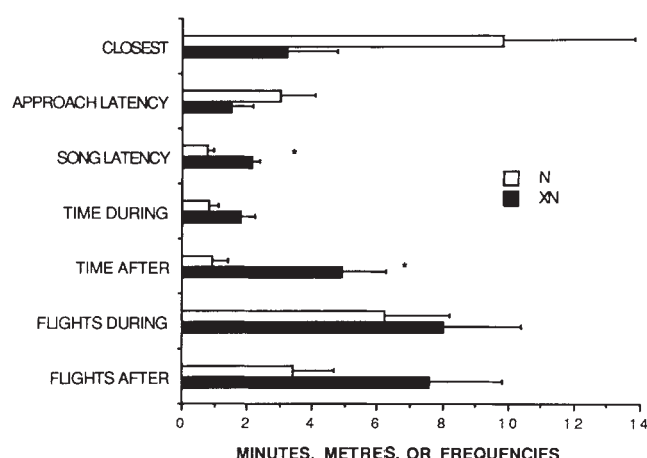


FIG. 4 Average responses and standard errors for male hooded warblers responding to N and XN playbacks of the previous year's neighbours ($N = 5$). Responses measured are the same as those described in Fig. 3. * $P < 0.05$, Wilcoxon matched-pairs test (1-tailed).

remembered their individual neighbours' songs from year to year.

Of 17 males with mapped territories in 1988, seven returned on different days in 1989. Of these, five had territories with at least two contiguous neighbours. On the day of his return, as detected by daily surveys of the study area, I presented each male with a 3-min tape of a neighbour's songs from the previous year at each of two locations, 15–20 m inside a correct and an opposite boundary from the previous year. Playbacks at the two locations took place one hour apart, with the order of presentation alternated (three subjects heard XN first, two heard N first); otherwise procedures followed those described above. Only one male had a neighbour in an adjacent territory at the time of playback.

If males remembered only former boundaries, I expected no difference between responses to N and XN presentations. But if males remembered both former neighbours' songs and their proper locations, I expected a greater response to XN playbacks,

as these might indicate a former neighbour attempting to expand his territory.

All differences in measured responses to N and XN playbacks resembled those in the 1988 experiment (Figs 3 and 4). A one-way analysis of variance on the composite scores derived from principal component analysis confirmed that males responded more to XN playbacks ($F_{1,8} = 8.017$, $P < 0.021$). I thus conclude that hooded warblers not only learned to associate different neighbours' songs with accustomed locations, but also remembered and recalled these associations after eight months without singing.

Recognition of neighbours, as demonstrated by a stronger response to XN playback, could be explained by habituation. But hooded warblers returning from migration had not heard neighbours' singing for 8 months, yet they responded more strongly to XN playbacks. This finding supports a previous report that recognition of neighbours involves associative learning, rather than habituation²³.

Long-term recognition of individual neighbours might provide immediate benefits in reproduction. Establishment and defence of territorial boundaries take time that males could spend attracting mates. If males with familiar neighbours can spend more time courting females, it would benefit them to return to their former territories and to remember neighbours²⁴. It thus seems plausible that long-term memory and recall of individual neighbours might represent an evolutionary adaptation in cognitive abilities in migratory species like the hooded warbler. □

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BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra

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BRAIN-derived neurotrophic factor (BDNF), present in minute amounts in the adult central nervous system¹, is a member of the nerve growth factor (NGF) (ref. 2) family, which includes neurotrophin-3 (NT-3) (refs 3–5). NGF, BDNF and NT-3 all support survival of subpopulations of neural crest-derived sensory neurons^{3–5}; most sympathetic neurons are responsive to NGF (ref. 2), but not to BDNF^{1,6,7}; NT-3 and BDNF, but not NGF (ref. 6), promote survival of sensory neurons of the nodose ganglion^{3–8}. BDNF, but not NGF, supports the survival of cultured retinal ganglion cells⁹ but both NGF and BDNF promote the survival of septal cholinergic neurons *in vitro*^{10,11}. However, knowledge of their precise physiological role in development and maintenance of the nervous system neurons is still limited. The BDNF gene is expressed in many regions of the adult CNS^{12–14}, including the striatum¹². A protein partially purified from bovine striatum, a target of nigral dopaminergic neurons, with characteristics apparently similar to those of BDNF, can enhance the survival of dopaminergic neurons in mesencephalic cultures¹⁵. BDNF seems to be a trophic factor for mesencephalic dopaminergic neurons, increasing their survival, including that of neuronal cells which degenerate in Parkinson's disease. Here we report the effects of BDNF on the survival of dopaminergic neurons of the developing substantia nigra.

Figure 1a illustrates the typical appearance of dissociated ventral mesencephalic cells after 8 days in culture. Using serum-free growth medium, few cells of fibroblast morphology were evident and virtually no astroglial cells were detected using antibodies to glial fibrillary acidic protein¹⁶. To visualize dopaminergic neurons in these cultures, immunocytochemical staining was carried out with monoclonal antibodies to tyrosine hydroxylase, a marker for dopaminergic neurons (TH; Fig. 1b–d). As expected, the number of TH-positive (TH+) neurons was small (arrow, Fig. 1b) and varied, representing 0.1–0.5% of the cells plated. In control cultures there was a gradual decline in the number of TH+ cells after the first 2–3 days *in vitro*. After 8 days, for example, the number of TH+ cells in control cultures was only 25% of that found in similar cultures at day 2 (Fig. 2). But in cultures treated with BDNF the loss of TH+ cells was greatly reduced (Fig. 2, and Table 1). The number of TH+ cells in BDNF-treated cultures was five times that in controls after 8 days in culture (Fig. 2), and the effect of BDNF was most pronounced when it was added at the start of the experiment and replenished every second day. But addition of even a single dose of BDNF after 24 h (Table 1, column 2) produced more than three times the number of TH+ neurons than in untreated controls when examined 8 days later (9 days *in vitro*). As previously reported^{15,17}, NGF (50 ng ml⁻¹) had no effect on the number of TH+ cells (data not shown). There was no effect of BDNF on the survival of the other neurons in these cultures, as the overall number of neurons after 6 days in culture was similar in control and BDNF-treated cultures (control, 27,618 ± 2,472; BDNF, 27,818 ± 1,016; cells cm⁻², *n* = 5).

That BDNF reduced the loss of TH+ cells in mesencephalic cultures suggested that either it acts directly to sustain survival

TABLE 1 Effect of time of addition of BDNF on numbers of TH+ neurons

Days in culture	TH+ cells per dish			
	No BDNF	+BDNF at 1 day	+BDNF at 4 days	+BDNF at 6 days
5	72	148	90	—
7	34	120	82	60
9	27	100	57	50

Delayed addition of BDNF does not increase the number of TH+ neurons to the same extent as when BDNF is added at 1 day *in vitro*. Cultures were prepared as described in the legend to Fig. 1, with the exception of the time of addition of BDNF. All cultures were switched to serum-free medium after 1 day, and BDNF (50 ng ml⁻¹) was added as a single addition either at this time or after 4 or 6 days *in vitro*. After 5, 7 and 9 days in culture the number of TH+ neurons was determined in duplicate cultures. In this experiment a single addition of BDNF after 1 day resulted in a threefold higher number of TH+ neurons at day 9. When BDNF was added after a four-day delay, at day 5, an increase in TH+ neurons over controls was seen at day 9, but was less than twofold, and this difference in TH+ cell number between treated and control cultures did not increase further with longer time in culture (data not shown). Results are expressed as the average of duplicate determinations.

of dopaminergic neurons, or it upregulates the expression of a dopaminergic phenotype (such as TH immunoreactivity) in neurons that do not necessarily require BDNF for survival. To distinguish between these possibilities, we determined the number of TH+ neurons in cultures in which BDNF addition was initially delayed for several days. In the experiment of Table 1, a single dose of BDNF was added either after 1, 4 or 6 days in culture and the number of TH+ cells was determined in duplicate cultures after 5, 7 or 9 days. When BDNF addition was delayed for 4 days, and the number of TH+ cells was then determined at 5, 7 or 9 days, fewer dopaminergic cells were seen in these cultures than in parallel cultures in which BDNF had been present from the start. Although delayed addition of BDNF could reduce some loss of TH+ neurons relative to controls, at any equivalent time, the number of TH+ neurons rescued was never as many as that achieved by maintaining the cultures in the presence of BDNF from the start. Extended culture did not increase the effect of delayed addition of BDNF (data not shown). Together these results argue against the possibility that BDNF increases TH gene expression in a fixed number of cells, but suggest that its addition to cultures at an early stage increases the survival of dopaminergic neurons that would otherwise be lost.

We do not know whether the effects of BDNF that we observed result from its direct action on dopaminergic neurons (through BDNF receptors on the TH+ neurons) or are mediated indirectly by its primary effect on another neuronal or nonneuronal population. The virtual absence of nonneuronal cells in these cultures tends to rule out BDNF acting indirectly through an effect on glial cells or fibroblasts. Binding data¹⁸ indicate that the low-affinity NGF receptor is also a low-affinity receptor for BDNF. We therefore reasoned that antibodies that recognize the NGF low-affinity receptor might also detect the low-affinity component of the BDNF receptor. By double labelling cultures with a rabbit antibody to TH and a mouse monoclonal anti-NGF receptor antibody¹⁹, we found that both in control and in BDNF-treated cultures almost 50% of TH+ neurons also showed NGF-receptor-like (NGF-R) immunoreactivity (94 NGF-R+ cells per 200 TH+ cells in one experiment counting random fields in three replicate dishes treated with BDNF for 6 days). The total number of NGF-R+ neurons was small, similar to the total number of TH+ cells. Thus, the colocalization of the NGF-BDNF low-affinity receptor to a large percentage of the TH+ neurons, argues in favour of a direct action for BDNF on dopaminergic neurons, especially as NGF itself has no effect on these neurons.

Dopaminergic neurons are selectively vulnerable to

FIG. 1 Phase-contrast (a) and bright-field photomicrographs (b–d) of dissociated cultures of E14 rat ventral mesencephalic cells stained after 6 or 8 days in culture with monoclonal antibodies to tyrosine hydroxylase (TH). a, b, Phase-contrast and bright-field photographs of the same field show the low abundance of TH+ neurons in typical cultures. Only a single TH+ neuron (arrows) is seen in a field containing many phase-bright neurons with long neurites. Few nonneuronal cells are evident, by morphology or by staining for GFAP (not shown). Scale bar, 100 μ m. c, d, Higher magnification photomicrographs of 6-day cultures of E14 rat ventral mesencephalic cells showing varied morphologies of TH+ neurons, some with extensive neuritic growth and extremely elaborate growth cones. Scale bar, 25 μ m.

METHODS. All cultures were prepared from the ventral mesencephalon dissected from 14-day-old embryonic rats (E14). Typically, pooled tissue from two or three litters of rat embryos from timed-mated Sprague-Dawley rats was trypsinized (0.125%; Worthington) in F12 medium (Gibco) for 20 min at 37 °C. After washing in growth medium containing 7.5% FCS, the tissue was centrifuged at low speed for 5 min and the pellet was dissociated by trituration. After allowing 1–2 min for nondispersed cell clumps to settle, the single-cell suspension was seeded onto 35-mm dishes (pre-coated with polyornithine and laminin⁶) containing growth medium to give a density of 5×10^4 cells cm^{-2} . After an overnight incubation in MEM medium supplemented with glutamine (2 mM), glucose (6 mg ml^{-1}), penicillin G (0.5 U ml^{-1}), streptomycin (5 μ g ml^{-1}) and FCS (7.5%) to allow cell attachment, cells were cultured in the presence or absence of BDNF in a serum-free, defined medium as described previously³², except that insulin was included at 20 ng ml^{-1} . To visualize dopaminergic cells, cultures were fixed with 4% paraformaldehyde, washed extensively, permeabilized with 0.02% saponin in Sorensen's buffer with 1.5% horse serum and stained with a mouse monoclonal antibody to rat TH (Boehringer-Mannheim). Primary antibody binding was visualized using a Vectastain ABC kit (Vector Labs).

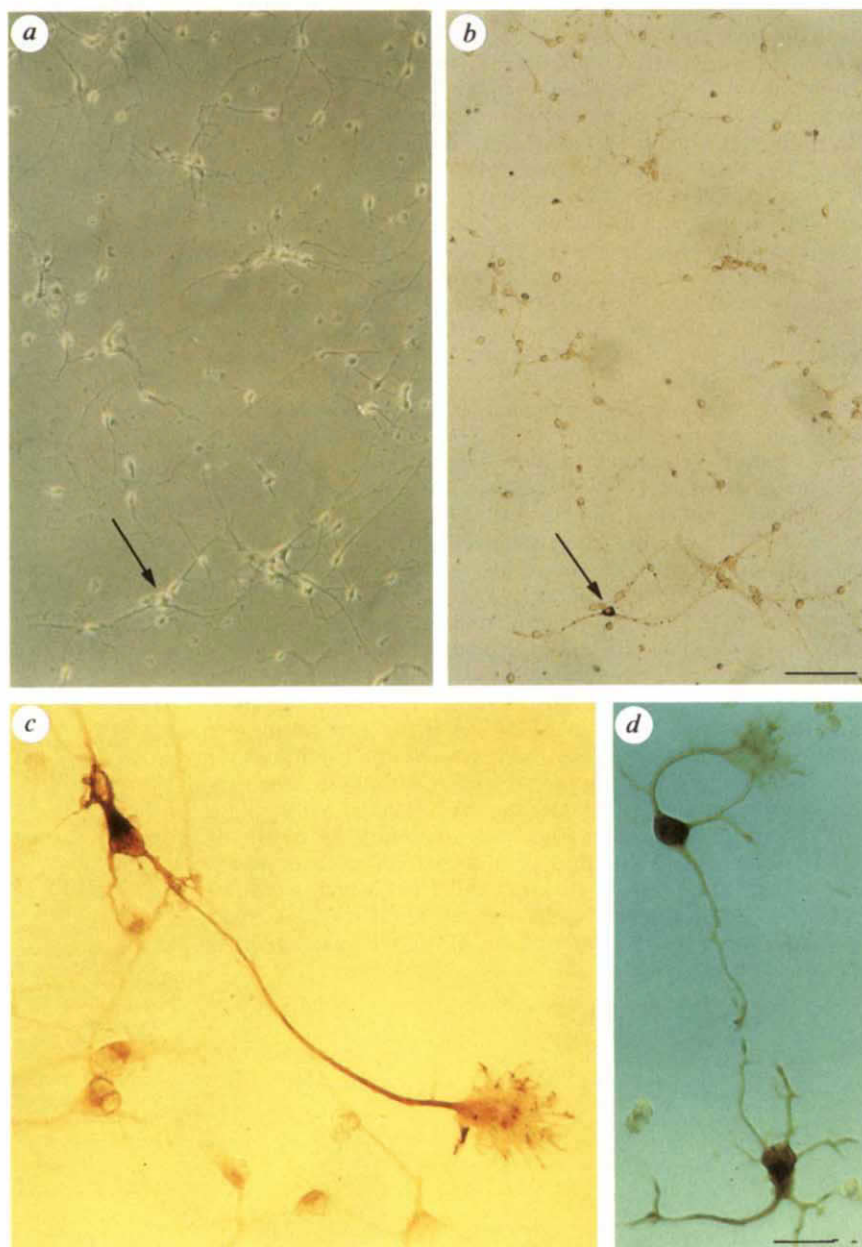
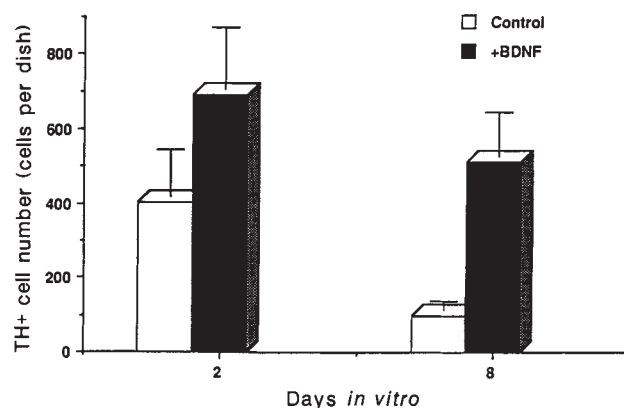


FIG. 2 Comparison of the number of TH immunoreactive neurons in cultures of E14 rat ventral mesencephalon maintained in the presence (solid bars) or absence (open bars) of BDNF.

METHODS. Cultures were established as described in Fig. 1. In BDNF-treated cultures, BDNF (50 ng ml^{-1}) was added on the day of plating and then replenished after 1, 3, 5 and 7 days *in vitro*. After 2 and 8 days *in vitro*, replicate cultures of control and BDNF-treated cells were fixed and processed for TH immunoreactivity. Results are the mean $\pm$ s.e.m. of quadruplicate determinations at each time point. Experiments were carried out either with BDNF purified from pig brain⁸ or with recombinant human BDNF, purified from the conditioned medium of a CHO cell line (DGZ1000-B-3-2.5; G.D.Y., D. Gies and S.P.S., manuscript in preparation) stably transfected and amplified for BDNF production. Recombinant hBDNF was purified as follows. Supernatant (50 ml) from the DGZ-1000-B-3-2.5 cells cultured in roller bottles was collected and dialysed against 6 M urea. The dialysed supernatant was then combined with ampholines (pH 3.5–10, BioRad) and run in a preparative isoelectric focusing chamber for 4.5 h. Fractions were collected, dialysed against 25 mM sodium phosphate (pH 7.6) using dialysis tubing which had been prewashed in BSA (0.5 mg ml^{-1}) to prevent nonspecific absorption. Fractions were assayed for neurite outgrowth promoting activity towards embryonic (E8) chick dorsal root ganglion explants. Active fractions were pooled and, by SDS-PAGE and subsequent silver staining³³ on a 8–18% gel, revealed a faint band at 68K corresponding to BSA and a single band at about 12K corresponding to that observed previously for purified porcine



BDNF (ref. 1; data not shown). In comparative bioassays carried out on chick dorsal root, nodose and sympathetic ganglion explants the specific activity and neuronal specificity of purified recombinant BDNF from CHO cells was similar to that of purified porcine BDNF. From cloning of the human BDNF gene³⁴, the deduced amino-acid sequence of mature human BDNF is shown to be identical to that of porcine BDNF.

neurotoxic agents such as 6-hydroxydopamine and MPP⁺ (1-methyl-4-phenylpyridinium; the active metabolite of MPTP, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine)²⁰. Having shown that BDNF increases the survival of dopaminergic neurons in cultures of embryonic rat ventral mesencephalon, we examined whether BDNF treatment could also reduce the susceptibility of these neurons to MPP⁺ toxicity. Exposure of 4-day control cultures or NGF-treated cultures to 1 μ M MPP⁺ for 48 h resulted in a loss of over 75% of the TH⁺ neurons, in agreement with previous studies^{21–23}. When cultures were treated with BDNF for 1 day (during the 4th day in culture) before exposure to MPP⁺, the loss of TH⁺ neurons was reduced to less than 32% (Fig. 3). But neither NGF nor basic fibroblast growth factor (bFGF; data not shown) showed any effect against MPP⁺ neurotoxicity.

Several reports have documented the stimulation of maturation and/or enhancement of survival *in vitro* of mesencephalic dopaminergic neurons by either target-derived extracts or various growth factors^{15,17,24–28}. There has been no report of a fully purified molecule directly and selectively supporting the survival of TH⁺ neurons in the absence of glial cells or enhanced cell division. In view of our results, the neurotrophic factor partially purified from bovine striatum by Dal Toso *et al.*¹⁵ could have been BDNF, although its identification as a small basic protein is also compatible with its being bFGF.

Although there are several reports^{17,28} of bFGF promoting survival of mesencephalic dopaminergic neurons, it has been suggested that the effects of bFGF are indirect, requiring the presence of glial cells¹⁷. Under our tissue culture conditions we observed that bFGF (10 ng per ml bovine brain FGF; Boehringer-Mannheim) did not counter the toxic effect of MPP⁺ (data not shown). This suggests that the prevention by bFGF of MPTP-induced decreases in dopamine and TH levels in mice²⁹ may require the presence of other cell types which were not present in our cultures. Application of bFGF *in vivo* could stimulate the synthesis and release of BDNF, among other neurotrophic activities.

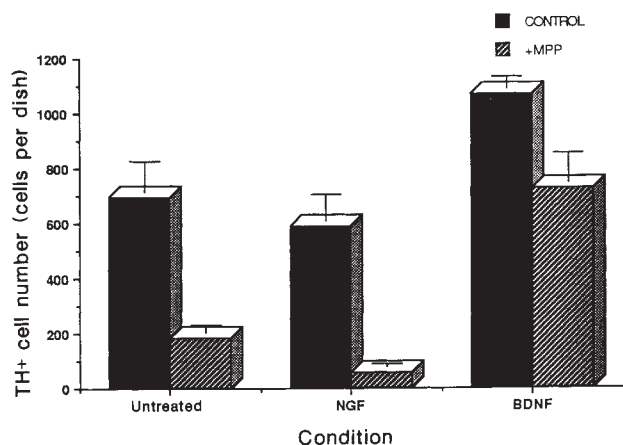


FIG. 3 BDNF protects cultured nigral dopaminergic neurons from the neurotoxic effects of MPP⁺.

METHODS. Cultures were established from E14 rat embryos as described in Fig. 1. After 24 h *in vitro* the culture medium was changed to the serum-free formulation. After 3 days in culture the cultures were divided into three groups of six 35-mm dishes. One group was kept as a control group, and the others received either mouse NGF, 50 ng ml⁻¹, or BDNF, 50 ng ml⁻¹. Cultures were kept for a further 24 h, before three dishes in each group were exposed to 1 μ M MPP⁺ (Research Biochemicals, Natick, Massachusetts) for 48 h. At the end of the experiment, 6 days in total, all plates were processed for TH immunoreactivity and the number of TH⁺ cells was determined in each group. For each pair of bars the data presented represent the number of TH⁺ neurons counted in replicate cultures maintained with or without MPP⁺ for 48 h. All values are the mean $\pm$ s.e.m. of triplicate cultures.

That BDNF markedly reduces the cytotoxicity of MPP⁺ *in vitro* is of clinical interest. Both in humans and nonhuman primates, MPTP can cause selective destruction of nigral dopaminergic neurons, and the acute onset of parkinsonian symptoms^{30,31}. Thus the availability of a suitable animal model, together with a fully characterized neurotrophic factor, provides a rationale for animal experiments to determine whether BDNF can protect dopaminergic neurons from the neurotoxic effects of either MPTP or 6-hydroxydopamine. Such studies may lead to a novel therapeutic approach for parkinsonism. □

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Determination of the subunit stoichiometry of a voltage-activated potassium channel

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THE voltage-activated K⁺, Na⁺ and Ca²⁺ channels are responsible for the generation and propagation of electrical signals in cell membranes. The K⁺ channels are multimeric membrane proteins formed by the aggregation of an unknown number of independent subunits^{1–3}. By studying the interaction of a scorpion toxin with coexpressed wild-type and toxin-insensitive mutant Shaker K⁺ channels, the subunit stoichiometry can be determined. The Shaker K⁺ channel is found to have a tetrameric structure. This is consistent with the sequence relationship between a K⁺ channel and each of the four internally homologous repeats of Na⁺ and Ca²⁺ channels^{4–6}.

The scorpion toxin charybdotoxin (CTX) and its homologues inhibit K⁺ channels in a simple bimolecular fashion^{7–11}. The

Shaker K^+ channel is inhibited by CTX and amino-acid residues on the channel that affect the toxin interaction have been identified¹¹⁻¹³. Figure 1 shows a toxin inhibition curve for the wild-type channel and for a point mutant, D431N. Compared with the wild-type channel, the mutant is only weakly sensitive to toxin: the inhibition constant is increased by around 250-fold. Figure 1 also shows an inhibition curve for channels produced by a 1:1 mixture of wild-type and mutant complementary RNAs injected into *Xenopus* oocytes. The result is initially surprising because the channels are quite sensitive to toxin. Only a small fraction of the channels in the mixing experiment can have the toxin insensitivity of the D431N mutant channel, suggesting that the wild-type phenotype is 'dominant'. This outcome is a reflection of the way in which the toxin molecule interacts with a multisubunit ion channel receptor.

Consider what can happen when wild-type and mutant channels are coexpressed. If the ion channel has n subunits, then a distribution of unique channels will result. Some channels will

have n wild-type subunits, some will have n mutant subunits, and many will have both subunit types; each channel species will presumably have a different toxin sensitivity. The overall unblocked fraction, U_{mix} , at a toxin concentration $[T]$ is given by the sum of the unblocked fractions contributed by each species i :

$$U_{\text{mix}} = \sum_{i=0}^n F_i \left(\frac{K_i}{K_i + [T]} \right) \quad (1)$$

where K_i is the inhibition constant for the i th species and F_i is the fraction of channels that are i -type. If we assume that wild-type and mutant subunits are expressed with equal efficiencies and that subunit aggregation is random then F_i is given by:

$$F_i = \binom{n}{i} f_{\text{mut}}^i f_{\text{wt}}^{(n-i)} \quad (2)$$

where f_{wt} and f_{mut} are the fractions of wild-type and mutant subunits and n is the subunit stoichiometry. Equation (2) gives the order-independent fraction of channels that have i mutant subunits and $(n-i)$ wild-type subunits.

Equation (2) is valid only if wild-type and mutant channels express equally and aggregate randomly. This assumption seems to be reasonable because the channels appear to differ only in their toxin sensitivity; they express functionally to the same extent and their gating and single-channel conductance properties are essentially identical (Fig. 2). These observations argue that the point mutation probably does not affect subunit aggregation and that f_{wt} and f_{mut} can be controlled by injecting known ratios of RNA.

The relatively high toxin sensitivity observed for the 1:1 mixture in Fig. 1 is expected if the hybrid forms having at least one wild-type subunit are significantly more sensitive to toxin than are channels containing n mutant subunits. To assess the sensitivity of the hybrid channels, equations (1) and (2) can be combined as follows:

$$U_{\text{mix}} = R + f_{\text{mut}}^n \left(\frac{K_n}{K_n + [T]} \right) \quad (3)$$

In equation (3), R is the unblocked fraction due to all species containing one or more wild-type subunits (equation (1) with index $i = 0$ to $n-1$) and the remaining term is the unblocked fraction due to the fully-mutant (n mutant subunits) form. The inhibition constant for the fully-mutant channel, K_n , can be determined independently by measuring toxin inhibition of mutant channels expressed alone in oocytes. In this case the unblocked fraction, U_{mut} , is given by

$$U_{\text{mut}} = \frac{K_n}{K_n + [T]} \quad (4)$$

TABLE 1 Mixtures of wild-type and mutant subunits predicts a K^+ clonal subunit stoichiometry of four

f_{mut}	U_{mix}	$[\ln \{f_{\text{mut}}\}]^{-1} [\ln \{(U_{\text{mix}})/(U_{\text{mut}})\}]$
0.9	0.49 ± 0.012	3.8 ± 0.3
0.8	0.32 ± 0.024	3.7 ± 0.3

The fraction of unblocked current at 400 nM toxin, U_{mix} , was determined in oocytes injected with either 90% ($f_{\text{mut}} = 0.9$) or 80% ($f_{\text{mut}} = 0.8$) mixtures of D431N RNA. The values shown are the mean $\pm$ s.e.m. of seven to eight separate measurements. The unblocked fraction for the pure D431N mutant channel at 400 nM toxin, U_{mut} , is 0.73 ± 0.01 (eight determinations). When the numbers for either mixture are substituted into the expression on the left hand side of equation (5), a number close to four is obtained. There is an important reason why the RNA mixtures in this study are weighted heavily towards the mutant channel. These mixtures favour a small R term (equations (4) and (5) in text) by shifting the channel population towards the fully mutant form. Therefore, high ratios of mutant to wild-type RNA and high concentrations of toxin are used to achieve a good estimate of n .

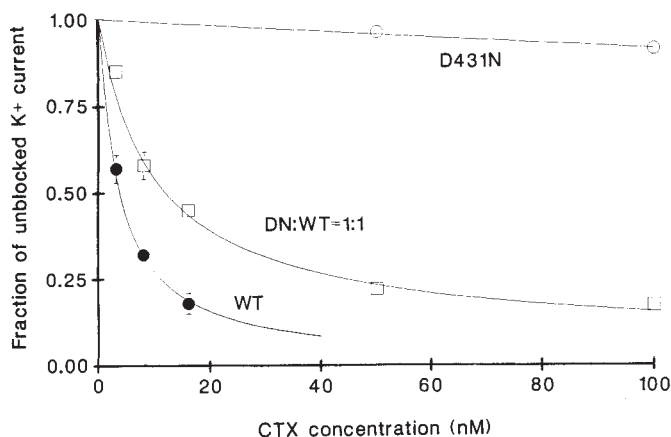
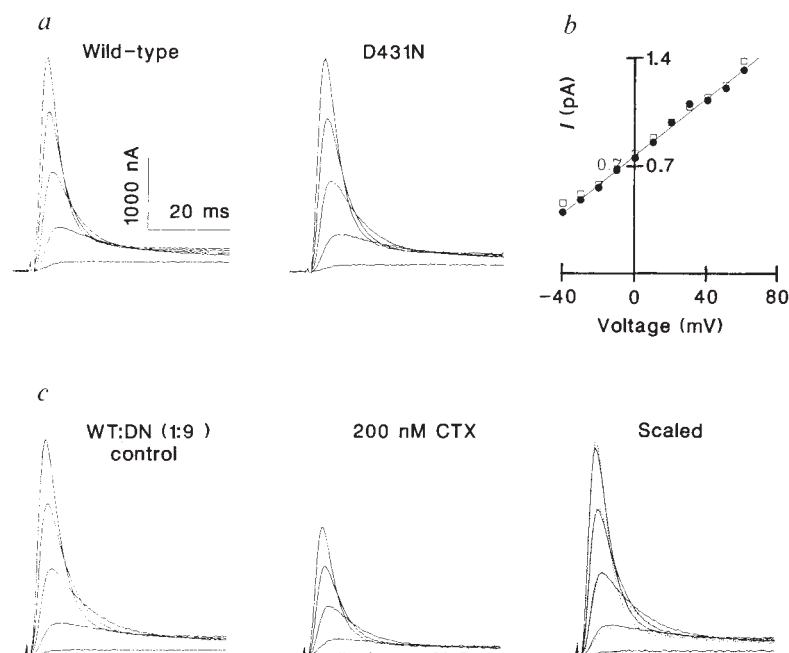


FIG. 1 A 1:1 mixture of wild-type and weakly toxin-sensitive mutant K^+ channels results in currents that are quite toxin sensitive. The fraction of unblocked K^+ current, U , is plotted as a function of toxin concentration for wild-type Shaker K^+ channels (●), for point mutant D431N (aspartate 431 replaced by an asparagine) (○), and for a 1:1 mixture of the wild-type and mutant (□). The data for wild-type and mutant are fitted according to equation (4) with $K_i = 3.7$ nM (wild-type) and 1,020 nM (mutant). The data for the 1:1 mixture are fitted according to equation (1) ($i = 0$ to 4). F_i is given by equation (2) when $n = 4$ and $f_{\text{wt}} = f_{\text{mut}} = 0.5$. The K_i s (in nM) are ($i = 0$ to 4 respectively) 3.7, 6, 12, 19, 1,020.

METHODS. All experiments were carried out using the Shaker H4 cDNA clone¹⁹. The point mutation D431N was produced using oligonucleotide-directed mutagenesis performed with the *dut⁻ ung⁻* selection scheme of T. A. Kunkel²⁰ and confirmed by dideoxysequencing²¹. RNA was prepared by linearization with *Hind*III followed by transcription with T7 polymerase. Tritiated uracil was incorporated during the transcription reaction to allow accurate determination of the concentration. Transcript, at a concentration of 0.6 or 1.0 mg ml⁻¹, was stored at -80°C . Mixtures of RNA from the wild-type and mutant clones were prepared using a calibrated pipette. Because of the importance of knowing the uncertainty in the mixture ratio, RNA was transcribed and mixtures were prepared in triplicate. Therefore, the uncertainty in the mixture ratio is built into the scatter in the data. *Xenopus* oocytes were prepared and injected as previously described¹¹. K^+ currents were recorded with a two-microelectrode voltage clamp (Axoclamp; Axon Instruments) using a P/4 leak subtraction²². The bath solution in all experiments contained (in mM) 96 NaCl, 2 KCl, 0.3 CaCl₂, 1 MgCl₂ and 5 HEPES buffer, pH 7.6. On addition of toxin to the perfusion medium currents were measured periodically, generally once per min, until equilibrium block was achieved. To maintain small currents and good voltage clamp, oocytes were studied within the first few days following RNA injection, the interpulse membrane potential was held between -80 mV and -50 mV to inactivate partially the Shaker K^+ channels, and depolarizing test pulses were made only up to 0 mV. Isochronal current amplitudes were measured between 6 ms and 15 ms into a 50-ms pulse. CTX was prepared as described⁸.

FIG. 2 Wild-type and D431N mutant Shaker K^+ channels are very similar. *a*, K^+ currents were recorded in oocytes two days after RNA injection. The holding potential was -60 mV and test potentials were between -40 mV and 0 mV for a duration of 50 ms. The current scale bar corresponds to 940 nA for D431N. *b*, Single-channel current for the wild-type ($\bullet$) and mutant ($\square$) channels are plotted against membrane voltage. Currents were recorded from inside-out excised patches containing fewer than five channels. The pipette solution was (in mM), 100 NaCl, 2 KCl, 5 MgCl₂, 10 HEPES buffer pH 7.1 , and the bath solution 100 KCl, 5 MgCl₂, 5 EGTA, 10 HEPES buffer pH 7.1 . *c*, K^+ currents were recorded under the conditions described in *a* from an oocyte injected with a $1:9$ mixture of the wild-type (WT) and mutant RNA. The current scale bar corresponds to 864 nA. Currents were recorded after inhibiting the sensitive fraction with 200 nM toxin. The traces on the right show the weakly sensitive fraction scaled and superimposed on the control record.



If the hybrid channels in a mixing experiment are significantly more sensitive to toxin than the fully-mutant channel, then at high toxin concentrations R in equation (3) will approach zero. This seems to be the case and is best displayed graphically by plotting the unblocked fraction, U , as a function of the product of $[T] \times U$. According to a transformation of equations (3) and (4) (see legend, Fig. 3a), the slope of such a plot is the negative

reciprocal of the inhibition constant. Figure 3a shows data for the D431N mutant channel and for a $1:9$ mixture of wild-type and mutant. At high toxin concentrations the data points for the mixture approach a straight line which runs parallel to the data set for the pure mutant channel. This is expected if, at high toxin concentrations, the hybrid channels become blocked leaving only fully mutant channels. By measuring CTX inhibition

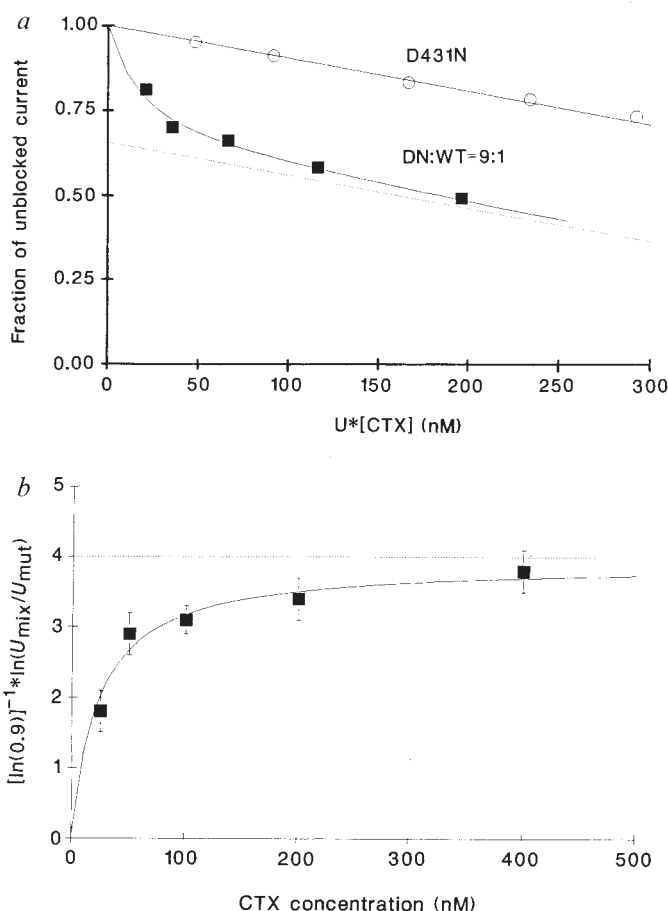


FIG. 3 *a*, The weakly toxin-sensitive fraction in a mixing experiment corresponds to the fully mutant form. The fraction of unblocked current, U , was determined at several toxin concentrations in oocytes injected with RNA from the D431N mutant clone (U_{mut}) and in oocytes injected with a $1:9$ mixture of wild type (WT) and mutant (U_{mix}). U is plotted as a function of the product of U and the toxin concentration ($[CTX]$, nM) according to a transformation of equation (3):

$$U_{mix} = \frac{-U_{mix}[T]}{K_n} + f_{mut}^n + R \left(\frac{K_n + [T]}{K_n} \right).$$

Equation (4) transforms to the above equation with $U_{mix} = U_{mut}$, $f_{mut} = 1$ and $R = 0$. The data points for the mutant channel fall on a straight line with negative slope inversely proportional to the inhibition constant ($1,020$ nM). At high toxin concentrations the data points for the mixture approach the same slope. Each point corresponds to the mean of three to eight measurements. The standard errors are smaller than the data points. The curve on the mixture data corresponds to $n=4$, $f_{mut}=0.9$, $K_n=1,020$ nM, $R = \sum_{i=n-2}^{n-1} F_i (K_i / (K_i + [T]))$ (F_i is given by equation (2)), and $K_{n-2} = K_{n-1} = 19$ nM. The values for K_{n-2} and K_{n-1} were chosen to minimize χ^2 when $n=4$. (The estimate of K_{n-2} is not very confident as F_{n-2} is small.) The same analysis using other possible values for n demonstrates that a subunit number of $n=4$ is 30 times more likely to produce the experimental results than is $n=5$ and is 10^5 times more likely than is $n=3$. Only the $i=n-2$ and $n-1$ hybrid channels are considered because when $f_{mut}=0.9$, $\sum_{i=n-2}^n F_i > 0.99$ for $n=3, 4$ or 5 . *b*, The toxin inhibition data shown in *a* were substituted into the expression on the left hand side of equation (5) and the resulting value is plotted as a function of the toxin concentration. At high toxin concentrations the data points converge to a value close to four. U_{mut} for 25 nM toxin was calculated from the known inhibition constant of $1,020$ nM. The curve corresponds to the theory (equation (5)) with the same parameters as in *a*.

of a mixture other than 1:9 (below), we will see that it is highly unlikely that any of the hybrid channels are weakly sensitive like the pure mutant channel.

We can now predict the subunit stoichiometry, n , of the Shaker K^+ channel. Combining equations (3) and (4) and taking logarithms yields:

$$\frac{1}{\ln(f_{\text{mut}})} \ln\left(\frac{U_{\text{mix}}}{U_{\text{mut}}}\right) = n - \frac{1}{\ln(f_{\text{mut}})} \ln\left(1 - \frac{R}{U_{\text{mix}}}\right) \quad (5)$$

Each quantity on the left-hand side of equation (5) is either known or is directly measurable. The value of the expression differs from n by a term whose magnitude depends on the value of R : as R approaches zero it will approach zero. Therefore, the expression will underestimate n , but will converge to n as U_{mix} and U_{mut} are determined at successively higher toxin concentrations. Figure 3b shows a plot of the values obtained upon substitution of U_{mix} (for $f_{\text{mut}} = 0.9$) and U_{mut} into the expression on the left-hand side of equation (5). At high toxin concentrations the data points converge to a value near 4, consistent with a tetrameric channel structure. The curves in Fig. 3 are the best fits generated from the theory ($n = 4$ provides a significantly better fit than does $n = 3$ or 5). Most importantly, however, the asymptotic value is independent of *a priori* assumptions about the value of n or specific values of K_i .

Equation (5) can be tested by asking whether the same value for n is obtained using a different ratio of wild-type to mutant subunits. Table 1 shows that a value close to four is also obtained with a 2:8 mixture ($f_{\text{mut}} = 0.8$). A very different value for U_{mix} is observed, but the difference is exactly compensated by the $1/\ln(f_{\text{mut}})$ term.

The results of this study provide evidence that the Shaker K^+ channel is a tetramer, on the basis of two assumptions. The first is that the wild-type and mutant subunits aggregate randomly. The similar properties and levels of expression of wild-type and mutant channels argue that this is plausible. The second assumption is that only the fully mutant form is weakly toxin sensitive in a mixing experiment. This is supported by the finding that the weakly sensitive fraction has the same toxin affinity as the pure D431N channel. However, if the hybrid channel containing only a single wild-type subunit (and $n-1$ mutant subunits) is weakly toxin sensitive like the fully mutant form, the data corresponding to $f_{\text{mut}} = 0.9$ are consistent with a 12-subunit channel. However, the data corresponding to $f_{\text{mut}} = 0.8$ are consistent with a 9-subunit channel. These two sets of data are simultaneously satisfied if the channel is a tetramer and all hybrid channels are toxin sensitive compared to the fully mutant form.

These results indicate that a single wild-type subunit confers upon the Shaker K^+ channel relatively high toxin sensitivity. This is initially surprising, but is explicable. The toxin is an asymmetric molecule¹⁴ that binds to a receptor with fourfold symmetry. The toxin must therefore combine with the channel in four indistinguishable ways. If a bound toxin molecule interacts strongly with only one of the subunits at position 431, then a mutation on a single subunit simply reduces by one the number of ways that the toxin molecule can combine with the channel. Therefore, even with three mutant and only one wild-type subunits, the toxin may be expected to block the channel with one quarter of the affinity ($K_i = 16$ nM). In fact, the inhibition constant for the channel containing a single wild-type subunit, estimated from the inhibition curve in Fig. 3a, is 19 nM.

It is interesting to compare the subunit stoichiometry of the Shaker K^+ channel (tetramer) with that of the nicotinic acetylcholine receptor ion channel (pentamer) and gap junction hemichannel (hexamer). The gap junction channel forms a very wide, nonselective pore that is about 16 Å in diameter^{15,16}. The nicotinic acetylcholine receptor ion channel is selective for cations over anions and has an intermediate pore diameter of about 6.5–7 Å¹⁷. The voltage-gated K^+ channels, however, are highly selective and have a narrow ion conduction pore¹⁷. As

was suggested by Unwin, there seems to be a simple relationship between the number of subunits in an ion channel and its pore diameter¹⁸. This correlation may be coincidental, but it is consistent with a crude model of an ion channel as a barrel with its subunits acting as the staves.

Note added in proof: It has recently been demonstrated that CTX prepared by a separate method inhibits the Shaker K^+ channel with low affinity²³. The high-affinity inhibitor used in this study may therefore be a CTX isoform (there are many). This does not influence the conclusions reached here about the channel structure. □

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Pentameric structure and subunit stoichiometry of a neuronal nicotinic acetylcholine receptor

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NEURONAL nicotinic acetylcholine receptors are members of a gene family of ligand-gated transmitter receptors that includes muscle nicotinic receptors, GABA_A receptors and glycine receptors^{1–4}. Several lines of evidence indicate that neuronal nicotinic receptors can be made up of only two subunits, an alpha (α) subunit which binds ligand, and a non-alpha (nα) or beta (β) subunit^{5–13}. The stoichiometry of each subunit in the functional receptor has been difficult to assess, however. Estimates of the molecular weight of neuronal nicotinic receptor macromolecules suggest that these receptors contain at least four subunits but probably not more than five^{5,12}. We have examined the subunit stoichiometry of the chick neuronal α₄/nα₁ receptor^{7,9} by first using site-directed mutagenesis to create subunits that confer different single channel properties on the receptor. Co-injection with wild-type and mutant subunits led to the appearance of receptors with wild-type, mutant and hybrid conductances. From the number of hybrid conductances, we could deduce the number of each subunit in the functional receptor.

The M2 region of muscle nicotinic acetylcholine receptor (nAChR) subunits participates in the channel pore^{14–18}, and charged residues on either side of M2 influence the rate of ion transport through the channel¹⁸. We investigated whether

To determine the effect of these charged residues on the rate of ion transport, we changed the lysine at position 260 of α_1 to a glutamic acid; we refer to this mutated subunit as α_1 E260. Figure 2*b* shows that the α_4/α_1 E260 receptor has a single channel conductance of 37.5 pS, almost twice that of wild-type α_4/α_1 receptors. In a parallel experiment, we changed the

METHODS. Nuclei of *Xenopus laevis* oocytes were co-injected with cDNAs for two subunits (in equal molar ratios)²³. α_4 and α_1 in *a*, α_4 and α_1 E260 in *b*; α_4 K266 and α_1 E260 in *c*. Single channel recordings were from outside-out patches at 22–24 °C with a List EPC-7 amplifier 2–5 days after injection²³. Currents were digitized and stored on tape and analysed with a DEC PDP 11/73 computer. Currents were filtered at 1.5 kHz and sampled at 10 kHz. Amplitude histograms were constructed by measuring the amplitude of individual openings. A computer routine that fits single channels with idealized square pulses was used to measure the single channel amplitudes. Only openings longer than 1 ms and in which a plateau had been reached were included. Low doses of ACh were used to ensure that the probability of opening was small in order to reduce simultaneous openings. The amplitude histograms in Figs 3 and 4 were modelled as the sum of gaussian distributions and a nonlinear curve-fitting routine was used to judge the best fit for the parameters²⁴. The means and variances of the largest and smallest distributions were constrained to the values obtained in the control experiments shown in Fig. 2, whereas the nonlinear fitting routine minimized the χ^2 to obtain estimations of the remaining parameters. In all outside-out patches, ACh currents ran down in 3–5 min after forming the patch. The bath solution was a modified Barth's medium (OR-2)²³, divalent-free, plus: 1 mM Ca^{2+} ; 1 μM atropine; 10 mM HEPES (pH adjusted to 7.3–7.4 with NaOH); and 50–100 nM ACh. The patch pipette contained: 80 mM KF; 20 mM KAc; 10 mM EGTA; 10 mM HEPES (pH adjusted to 7.4 with NaOH, final Na concentration was 2 mM).

M 2

To assess the number of α_4 subunits in the functional receptor, we co-injected complementary DNAs for α_4 and α_4 K266 together with $n\alpha_1$ E260 into oocytes. The rationale for this experiment is that in these oocytes some receptors will form that only

METHODS. Nuclei of *Xenopus laevis* oocytes were co-injected with cDNAs for two subunits (in equal molar ratios)²³. α_4 and α_1 in *a*, α_4 and α_1 E260 in *b*; α_4 K266 and α_1 E260 in *c*. Single channel recordings were from outside-out patches at 22–24 °C with a List EPC-7 amplifier 2–5 days after injection²³. Currents were digitized and stored on tape and analysed with a DEC PDP 11/73 computer. Currents were filtered at 1.5 kHz and sampled at 10 kHz. Amplitude histograms were constructed by measuring the amplitude of individual openings. A computer routine that fits single channels with idealized square pulses was used to measure the single channel amplitudes. Only openings longer than 1 ms and in which a plateau had been reached were included. Low doses of ACh were used to ensure that the probability of opening was small in order to reduce simultaneous openings. The amplitude histograms in Figs 3 and 4 were modelled as the sum of gaussian distributions and a nonlinear curve-fitting routine was used to judge the best fit for the parameters²⁴. The means and variances of the largest and smallest distributions were constrained to the values obtained in the control experiments shown in Fig. 2, whereas the nonlinear fitting routine minimized the χ^2 to obtain estimations of the remaining parameters. In all outside-out patches, ACh currents ran down in 3–5 min after forming the patch. The bath solution was a modified Barth's medium (OR-2)²³, divalent-free, plus: 1 mM Ca^{2+} ; 1 μM atropine; 10 mM HEPES (pH adjusted to 7.3–7.4 with NaOH); and 50–100 nM ACh. The patch pipette contained: 80 mM KF; 20 mM KAc; 10 mM EGTA; 10 mM HEPES (pH adjusted to 7.4 with NaOH, final Na concentration was 2 mM).

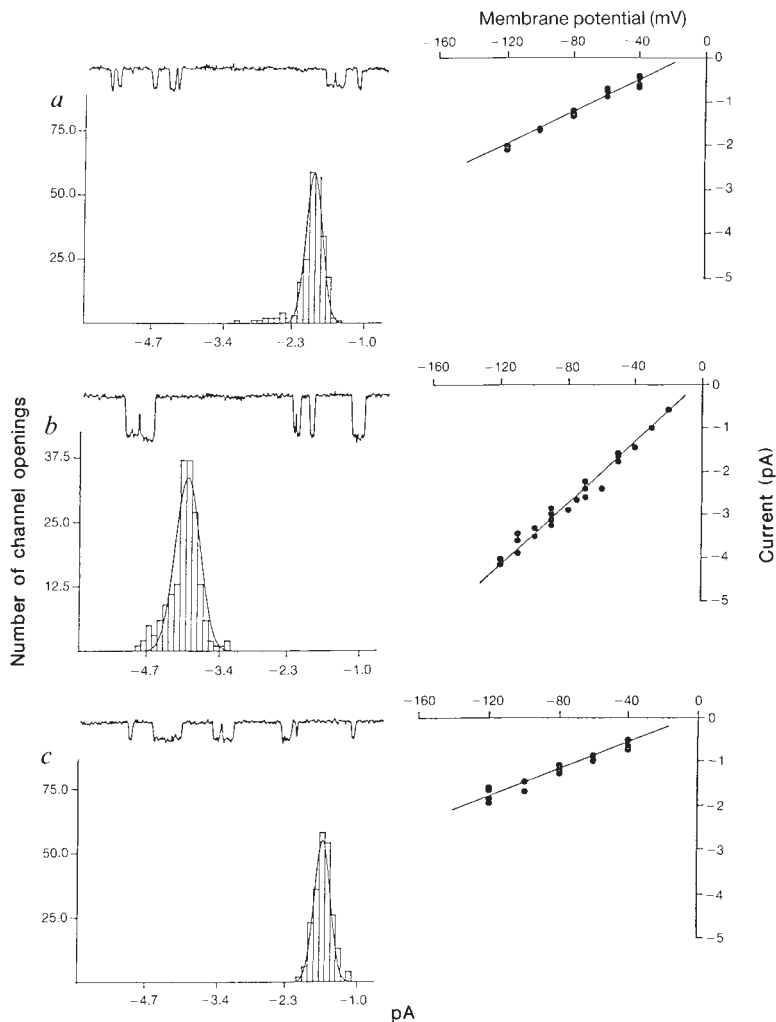
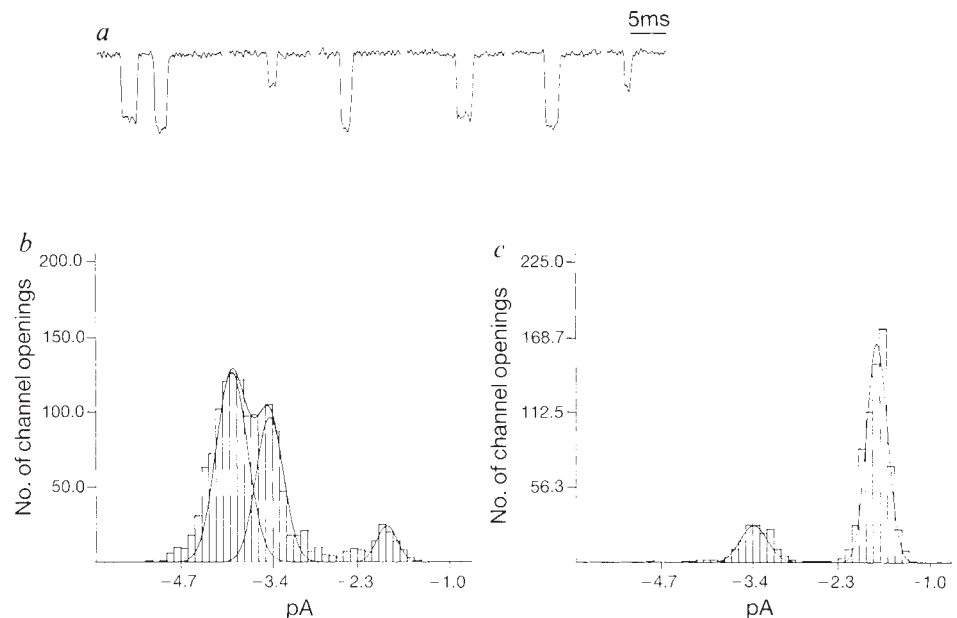


FIG. 3 Assembly of two α subunits in the functional receptor. Both α_4 and α_4 K266 were coinjected with α_1 into the same oocyte. *a*, Single channel records measured in an outside-out patch held at -120 mV and shows three receptors with different current amplitudes. *b*, Amplitude histograms of over 500 such openings. These histograms were best fitted as the sum of three gaussian distributions. The gaussian with a mean amplitude of -4.0 pA corresponds to that used to fit the α_4/α_1 E260 receptor in Fig. 2*b*. The gaussian with a mean of -1.85 pA corresponds to the gaussian used to fit the α_4 K266/ α_1 E260 receptor in Fig. 2*c*. The gaussian with a mean amplitude of -3.4 pA corresponds to receptors that have assembled with both α_4 and α_4 K266 together with α_1 E260. The amplitude histogram in *b* was from an oocyte co-injected with equal amounts of α_4 and α_4 K266. The amplitude histogram in *c* was from an oocyte co-injected with four times more α_4 K266 than α_4 .



incorporate α_4 subunits with α_1 E260 and others will form that incorporate α_4 K266 with α_1 E260. If, however, the number of α subunits in the functional receptor is >1 , then some receptors should incorporate both α_4 and α_4 K266 in the same receptor. On the basis of the effect of the charge near M2 on the conductance, the single channel conductance for these receptors should be between that of the α_4/α_1 E260 receptor and that of the α_4 K266/ α_1 E260 receptor (see Fig. 2). If the receptor assembles with N α subunits, then there should be $N-1$ receptors expressed in the oocyte membrane which have incorporated both α_4 and α_4 K266 subunits.

The results of coexpressing α_4 and α_4 K266 together with α_1 E260 are shown in Fig. 3. These co-injected oocytes express receptors with three different conductances, as illustrated by the amplitude histograms in Fig. 3*b*. These histograms were best fitted as the sum of three gaussian distributions. One gaussian with a mean of -4.0 pA corresponds to that used to fit the α_4/α_1 E260 receptor (see Fig. 2*b*). One gaussian with a mean of -1.8 pA corresponds to that used to fit the α_4 K266/ α_1 E260 receptor (see Fig. 2*c*). The remaining gaussian has a mean intermediate between the α_4/α_1 E260 receptor and the α_4 K266/ α_1 E260 receptor. The simplest interpretation of this, based on the effects of charged residues at the outer edge of M2 on the single channel conductance, is that it represents a hybrid receptor that has incorporated both α_4 and α_4 K266 subunits. This result was observed in all 18 patches from oocytes involving co-injection of α_4 and α_4 K266 with α_1 E260, and, as shown in Fig. 3*b* and *c*, the amplitude distribution could be

biased in favour of one receptor or the other by injecting differing amounts of cDNA for one subunit relative to the other. We conclude from this experiment that there are only two α subunits in the functional receptor. This is consistent with the results from the Hill coefficient measured in physiological dose-response experiments on the α_4/α_1 receptor⁷, and is identical to the two α subunit stoichiometry in muscle nAChRs²⁰.

The number of α_1 subunits was assessed in a similar fashion: we coinjected α_1 and α_1 E260 together with α_4 into oocytes

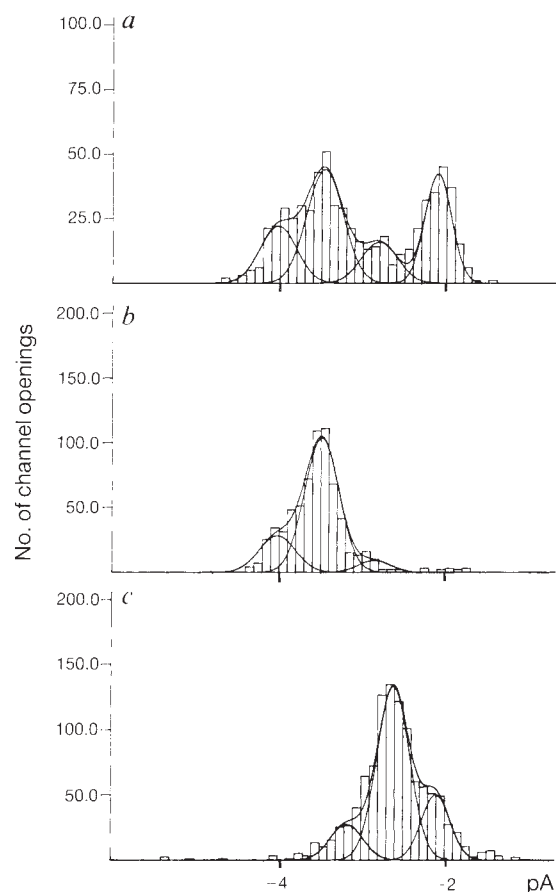


FIG. 4 The assembly of three α_1 subunits in the functional receptor. Both α_1 and α_1 E260 were co-injected with α_4 into the same oocyte. *a*, Results from an oocyte co-injected with equal amounts of α_1 and α_1 E260. This histogram was best fit as the sum of four gaussian distributions. The gaussian with a mean of -4.0 pA corresponds to the α_4/α_1 E260 receptor. The gaussian with a mean of -2.0 pA corresponds to the α_4/α_1 receptor. The gaussian with a mean of -2.7 pA corresponds to receptors that have assembled with two α_1 subunits and one α_1 E260 subunit. The gaussian with a mean of -3.5 pA corresponds to receptors that have assembled with one α_1 subunit and two α_1 E260 subunits. *b*, Results from an oocyte coinjected with 1.5 times more α_1 E260 than α_1 . In this histogram, the gaussian with the largest peak had a mean of -3.5 pA and corresponds to receptors that have assembled with one α_1 subunit and two α_1 E260 subunits. *c*, Results from an oocyte coinjected with 2.5 times more α_1 than α_1 E260. The main gaussian in this histogram had a mean of -2.7 pA and corresponds to receptors that have assembled with two α_1 subunits and one α_1 E260 subunit.

(Fig. 4). The amplitude histograms were best fitted as the sum of four gaussians (Fig. 4a). The gaussian with the largest mean amplitude (-4.0 pA) corresponds to the α_4/α_1 E260 receptor (see Fig. 2b). The gaussian with the smallest mean amplitude (-2.0 pA) corresponds to that used to fit the wild-type α_4/α_1 receptor (see Fig. 2a). The two intermediate gaussians had means of -2.7 pA and -3.5 pA. Using the same arguments based on charged residues at the outer portion of the M2 domain, the gaussian with a mean of -2.7 pA corresponds to hybrid receptors that have incorporated two α_1 and one α_1 E260 subunits into the functional receptor, whereas the gaussian with a mean of -3.5 pA corresponds to receptors that have incorporated one α_1 and two α_1 E260 subunits. Similar results to those in Fig. 4a were observed in 12 patches from oocytes co-injected with α_1 and α_1 E260. In addition, the amplitude distributions could be biased in favour of one receptor subtype by injecting differing amounts of cDNA for one subunit relative to the other (Fig. 4b and c). The oocyte analysed in Fig. 4b predominantly expressed a receptor subtype incorporating one α_1 subunit and two

α_1 E260 subunits, whereas the oocyte in Fig. 4c mainly expressed a receptor subtype that had incorporated two α_1 and one α_1 E260 subunits. Therefore, we conclude from this experiment that there are three α subunits in the functional neuronal nAChRs.

The results of this study show that functional neuronal nAChRs are pentameric complexes incorporating two α subunits and three α subunits. The approach taken here should also be useful in determining the stoichiometry of subunits for other ligand-gated receptors. Recently, several different α and α subunits for neuronal nAChRs have been identified^{6,9,10}. Our results indicate that functional neuronal nAChRs with different properties could assemble *in vivo* through the combination of different subunits. Therefore, it is conceivable that neurons may incorporate two different α subunits, coded for by different genes, or two or three different α subunits into the same receptor molecule, thereby increasing the possible combinations of functionally different nAChRs expressed in the nervous system. □

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Microtubule translocation in the cytokinetic apparatus of cultured tobacco cells

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IN higher plant cells, cytokinesis is achieved by new cross-wall formation mediated by the phragmoplast¹, a double ring of microtubules of opposite polarity, in which the short microtubules are arranged perpendicular to the equatorial plane of the phragmoplast with their plus ends interdigitating at the plane^{1,2}. The phragmoplast and its enclosed cell plate move out centrifugally until the mother cell divides. We report here results of a newly developed method using glycerinated cultured tobacco cells, which show that the equatorial region of the phragmoplast can translocate microtubules towards their minus ends concomitantly with tubulin polymerization at their plus ends. The translocation is induced effectively by GTP and less effectively by ATP, and is inhibited by the unhydrolysable nucleotide analogues GMP-PNP and AMP-PNP. Thus, the equatorial region of the phragmoplast seems to be associated with a mechanochemical enzyme that generates the force for microtubule translocation by hydrolysing GTP.

Cultured tobacco cells with phragmoplasts were treated with glycerol to permeabilize the plasma membrane³ and DTAF (dichlorotriazinylamino fluoroscein)-labelled tubulin was introduced into the glycerinated cells. Although cytokinetic progression was arrested by glycerination, an array of phragmoplast microtubules was well preserved in the glycerinated cells, as

judged by anti-tubulin immunofluorescence (Fig. 1f). As has been reported¹, there was no staining of the equatorial region where microtubules are interdigitating with their plus ends (Fig. 1f).

A bright fluorescent band appeared at the equatorial region of the phragmoplast in the glycerinated cells incubated with DTAF-tubulin (Fig. 1a), suggesting that DTAF-tubulin polymerizes onto the plus ends of pre-existing phragmoplast microtubules. Weak, broad fluorescence appeared at the distal portions of the phragmoplasts (Fig. 1b, c), suggesting that polymerization also occurs at the minus ends (Fig. 4a). The width of the bright band at the equatorial plane increased with time (Fig. 1a–c), and the distal, broad fluorescent bands moved away from the equatorial plane (Fig. 1b, c), suggesting that the microtubules are translocated towards their minus ends concomitantly with tubulin polymerization onto their plus ends. Microtubule translocation was more clearly shown by the experiment in which cells like the one in Fig. 1b were further incubated with unlabelled tubulin. The fluorescent band at the equatorial region is split in two by a newly formed, unlabelled band and each of the resultant pair of fluorescent bands is translocated away from the equatorial plane (Figs 1d, e; and 4b). Figure 2a shows that the unlabelled band appears after a short lag period, during which the interdigitating portions of fluorescent microtubules move apart, and increases its width with time. The rate of translocation seems to depend on the rate of polymerization when the tubulin concentration is lower than 0.5 mg ml^{-1} . The separation of the fluorescent band does not occur in the absence of unlabelled tubulin (Fig. 2a). But when the tubulin concentration is 0.5 mg ml^{-1} or higher, the rate of translocation seems to be limited by the activity of the microtubule-translocating system.

In the presence of GMP-PNP or AMP-PNP, phragmoplasts

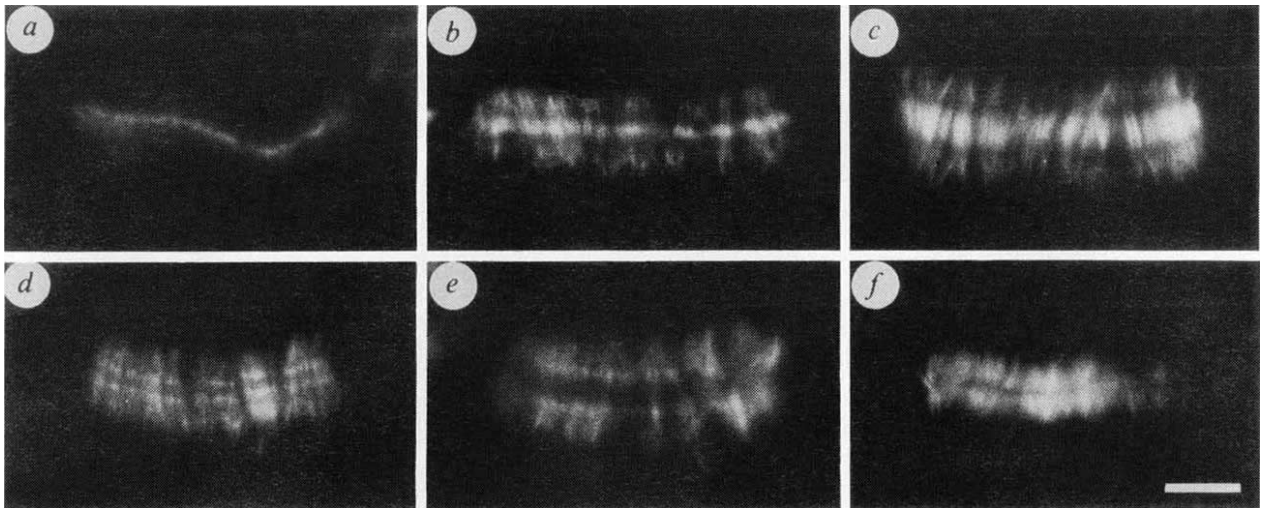


FIG. 1 Incorporation of exogenous tubulin into the phragmoplast. *a–c*, Glycerinated cells with phragmoplasts incubated with 0.5 mg ml^{-1} DTAF-tubulin for *a*, 5, *b*, 20 or *c*, 50 min. *d, e*, Cells like the one in Fig. 1*b* incubated further with 0.5 mg ml^{-1} unlabelled tubulin for *d*, 5 or *e*, 20 min. *f*, A phragmoplast in a glycerinated cell stained for microtubules with immunofluorescent anti-tubulin antibodies. Scale bar, $10 \mu\text{m}$.

METHODS. The cell cycle of tobacco BY-2 cells was synchronized⁸ and cells, about 80% of which were in anaphase or telophase, were treated with cell-wall digesting enzymes (1% Cellulase (Onozuka RS), 0.1% Pectolyase Y-23 in 0.4 M mannitol, pH 5.5) for 5 min then washed twice with 0.5 M mannitol solution. To permeabilize the plasma membrane, cells were suspended in 50 mM PIPES buffer (pH 7.0) containing 60% glycerol, 1 mM MgCl_2 , 5 mM EGTA and 1 mM dithiothreitol for 10 min at $4\text{--}10^\circ\text{C}$, and then for 0.5–2 h on ice. The plasma-membrane permeabilized cells prepared in this way are referred to as glycerinated cells. Just before use, glycerinated cells

were washed twice with PME-buffer (75 mM PIPES, pH 7.0 containing 1 mM MgCl_2 and 1 mM EGTA) and suspended in PME-buffer containing $5 \mu\text{M}$ taxol, 4 mM GTP and 15% glycerol. Bovine brain tubulin⁹ was labelled with dichlorotriazinyl-amino-fluorescein (DTAF)¹⁰. DTAF-labelled tubulin (DTAF-tubulin) in PME-buffer was added to the cell suspension and incubated at 30°C . In the experiments in which cells incubated with DTAF-tubulin were further incubated with unlabelled tubulin, the cell suspension with DTAF-tubulin was mixed with 10 vol PME-buffer containing $10 \mu\text{M}$ taxol, and the cells were collected by centrifugation and incubated with unlabelled tubulin as in the incubation with DTAF-tubulin. Tubulin polymerization did not require taxol, but polymerized microtubules did not withstand procedures necessary for changing the incubation medium in the absence of taxol. The density of cells during the incubation with exogenous tubulin was adjusted to $\sim 100 \mu\text{l}^{-1}$. Immunofluorescence was performed as in ref. 8.

incorporate DTAF-tubulin at the equatorial region, but the changes in the fluorescent pattern with time are distinct from those observed in their absence. The distal, weak fluorescent bands are not translocated by broadening of the fluorescent band at the equatorial region, but are overlaid by this band (compare Fig. 3*a* with Fig. 3*c, e*). The simple explanation of this observation is that GMP-PNP or AMP-PNP inhibit microtubule translocation without inhibiting tubulin polymerization. To visualize the effects of these drugs more clearly, the cells were incubated first with unlabelled tubulin in the presence of

GMP-PNP or AMP-PNP and then with DTAF-tubulin in the presence of GMP-PNP or AMP-PNP. DTAF-tubulin was incorporated at the sites distant from the equatorial region (Fig. 3*d, f*), indicating that the sites of addition of tubulin were separated from the equatorial region during the first incubation (Fig. 4*c*). This seems to be the result of polymerization of unlabelled tubulin onto plus ends of microtubules, the translocation of which was inhibited by GMP-PNP or AMP-PNP. In the absence of these drugs, a bright fluorescence appeared at the equatorial region, and weak, broad fluorescence appeared distal to the

FIG. 2 The rate of microtubule translocation in the phragmoplast. *a*, Dependency of the rate of microtubule translocation coupled with polymerization on the concentration of tubulin. Rates of translocation in the presence of 1 mg ml^{-1} ($\circ$), 0.5 mg ml^{-1} ($\bullet$), 0.25 mg ml^{-1} ($\square$) or 0.1 mg ml^{-1} tubulin ($\blacksquare$) or in its absence ($\triangle$) are shown. *b*, Dependency of the translocation of prepolymerized microtubules on nucleotide triphosphate. Rates of translocation in the presence of 4 mM GTP ($\circ$) or ATP ($\bullet$) are given. For each measurement more than 20 phragmoplasts were examined. Vertical bar, standard error.

METHODS. *a*, Glycerinated cells with phragmoplasts were incubated first with 0.5 mg ml^{-1} DTAF-tubulin together with 4 mM GTP for 20 min and then with unlabelled tubulin together with 4 mM GTP. At appropriate times, the cells were sampled and fixed with methanol at -15°C , and the width of the unlabelled band between two fluorescent bands was measured. Time 0 is the start of the incubation with unlabelled tubulin. *b*, Glycerinated cells with phragmoplasts were incubated with 0.5 mg ml^{-1} DTAF-tubulin together with 4 mM GTP for 20 min and then with 0.5 mg ml^{-1} unlabelled tubulin together with 3 mM GTP and 1 mM GMP-PNP for 30 min at 30°C . The cells were washed three times with 10 vol. of PME-buffer containing $10 \mu\text{M}$ taxol, and incubated with 4 mM GTP or 4 mM ATP in 75 mM PIPES buffer, pH 7.0, containing $10 \mu\text{M}$ taxol, 4 mM MgCl_2 , 1 mM EGTA and 1 mM DTT. The width of the unlabelled band between two fluorescent bands was measured. Time 0 is the start of the incubation with GTP or ATP. For further details, see Fig. 1 legend.

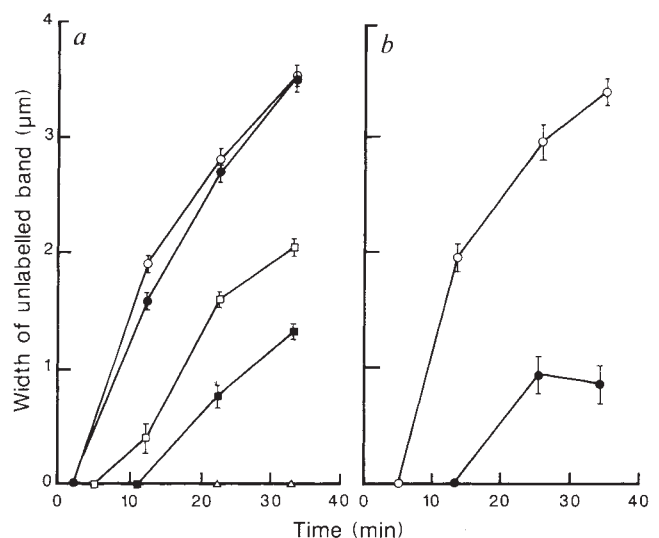
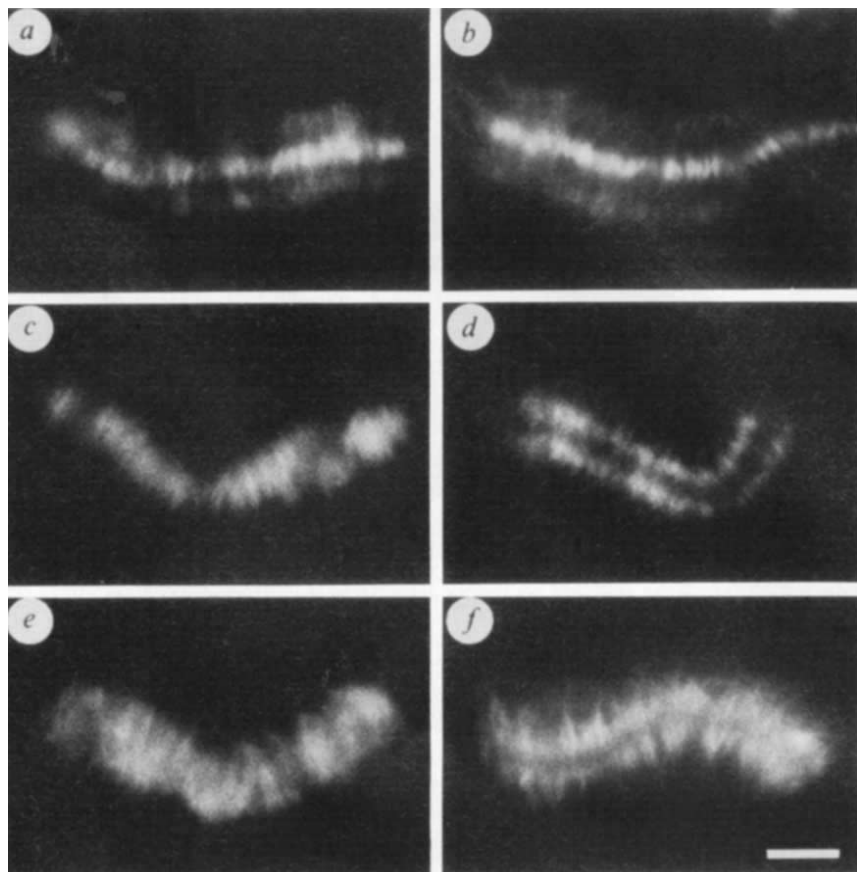


FIG. 3 Effects of GMP-PNP and AMP-PNP on incorporation of exogenous tubulin into phragmoplasts. *a, c, e*, Glycerinated cells with phragmoplasts incubated with 0.5 mg ml^{-1} DTAF-tubulin for 45 min. The incubation was carried out in the presence of *a*, 4 mM GTP, *c*, 2 mM GTP plus 2 mM GMP-PNP, or *e*, 4 mM GTP plus 0.1 mM AMP-PNP. *b, d, f*, Glycerinated cells with phragmoplasts incubated first with 0.5 mg ml^{-1} unlabelled tubulin for 30 min and then with 0.5 mg ml^{-1} DTAF-tubulin for 15 min. Both the first and the second incubations were carried out in the presence of *b*, 4 mM GTP, *d*, 2 mM GTP plus 2 mM GMP-PNP, or *f*, 4 mM GTP plus 0.1 mM AMP-PNP. Scale bar, $10 \mu\text{m}$.



broad unlabelled zones formed during the first incubation with unlabelled tubulin (Fig. 3*b*).

To examine whether microtubules that have polymerized in the presence of unhydrolysable analogues of GTP or ATP can be translocated by GTP or ATP, glycerinated cells were incubated first with DTAF-tubulin in the absence of such an analogue and then with unlabelled tubulin in the presence of GMP-PNP or AMP-PNP. After washing out free tubulin and analogue, the cells were incubated with GTP or ATP. When GMP-PNP was included, the fluorescent band at the equatorial region that had appeared during the initial incubation was split to two by an unlabelled band during incubation with GTP (Fig. 2*b*), giving a pattern like that shown in Fig. 1*d* or *e*. The result indicates that GTP causes the translocation of unlabelled microtubules that polymerize during incubation with GMP-PNP (Fig. 4*d*). This change in the pattern of fluorescence was not inhibited by the addition of hexokinase and glucose (data not shown). ATP also causes the separation of the fluorescent band, but is far less effective than GTP (Fig. 2*b*). The experiment in which AMP-PNP was used gave essentially the same results as those shown in Fig. 2*b*: GTP was far more effective than ATP. The finding that microtubule translocation is inhibited by GMP-PNP and is effectively induced by GTP strongly suggests that GTP is the preferable substrate of the mechanochemical enzyme for the translocation of phragmoplast microtubules. We cannot at this stage explain why AMP-PNP inhibits translocation in the presence of GTP.

Our results show that the equatorial region of the phragmo-

plast is associated with a specific activity that couples microtubule polymerization at the plus ends with microtubule translocation towards the minus ends. This microtubule translocation bears some resemblance to the sliding of microtubules in the isolated diatom spindle system⁴ and microtubule translocation in the microtubule-chromosome complex system⁵, but with a different mechanochemical enzyme: the phragmoplast system seems to use GTP, whereas the other two use ATP. GTP is

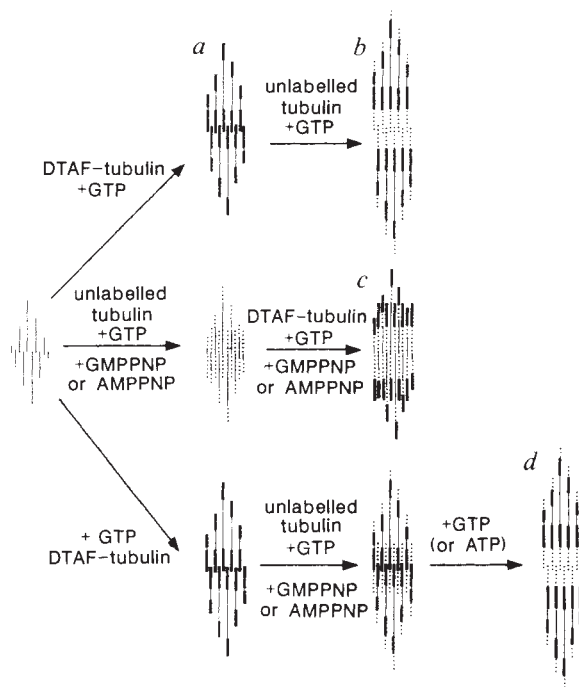


FIG. 4 Schematic illustration to explain fluorescence patterns shown in *a*, Fig. 1*b, c*; *b*, Fig. 1*d, e*; *c*, Fig. 3*d, f*; and *d*, Fig. 2*b*. Longitudinal cross-sectional views of one side of a ring-shaped mature phragmoplast are illustrated. — Preexisting microtubule; ···· polymerized unlabelled tubulin; — polymerized DTAF-tubulin.

thought to be the likely physiological substrate for dynamin, a force-producing microtubule-activated nucleotide triphosphatase in animal brain tissues^{6,7}.

The oppositely directed microtubule array in the phragmoplast seems to be essential for the accumulation of vesicles filled with cell-plate materials at the equatorial plane. The force-producing protein with GTPase activity at the equatorial region of the phragmoplast should be important in constructing such an array of phragmoplast microtubules. □

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The developmental gene *Knotted-1* is a member of a maize homeobox gene family

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THE *Knotted-1* (*Kn1*) locus is defined by several dominant gain-of-function mutations that alter leaf development. Foci of cells along the lateral veins do not differentiate properly, but continue to divide, forming outpocketings or knots. The ligule, a fringe normally found at the junction of leaf blade and sheath, is often displaced and perpendicular to its normal position^{1–3}. The phenotype is manifested in all cell layers of the leaf blade, but is controlled by a subgroup of cells of the inner layer⁴. Mutations result from the insertion of transposable elements⁵ or a tandem duplication⁶. We show that the *Kn1* gene encodes a homeodomain-containing protein, the first identified in the plant kingdom. Sequence comparisons strongly suggest that *Kn1* acts as a transcription factor. Here we use the *Kn1* homeobox to isolate other expressed homeobox genes in maize. The *Kn1* homeobox may permit the isolation of genes that, like animal and fungal counterparts⁷, regulate cell fate determination.

We have isolated complementary DNAs of the *Kn1* locus from maize seedling RNA using genomic DNA that was previously cloned by transposon tagging⁵. Reversion analysis had indicated that these sequences are closely associated with the *Kn1* gene^{5,6}. The sequence of the *Kn1* transcript (Fig. 1) was identified from cDNA sequences and primer extension assays (data not shown). The predicted transcript size of 1,635 base pairs (bp) agrees closely with the 1.7-kilobase (kb) messenger RNA identified in RNA blots (data not shown). The ATG at position 181 of the deduced transcription unit is probably the

translation start site for the *Kn1* protein as it is the first ATG codon, and is followed by one long open reading frame of 1,240 bp.

Comparison of the encoded polypeptide sequence with protein sequences in the Swiss Protein Database (release 14.0) using the FASTP program⁸ reveals limited but significant similarity to a variety of eukaryotic proteins that contain homeodomains⁹. This similarity is restricted to the amino-acid segments composing the homeodomains. The homeodomain is a 61-amino-acid motif which is postulated to bind DNA through the helix-turn-helix motif^{9–11}. An alignment of a portion of the *Kn1* amino-acid sequence with homeodomain sequences from various other eukaryotes is presented in Fig. 2a. The *Kn1* homeodomain is most similar to the homeodomains from the human Prl (pre-B cell leukaemia)^{12,13} and *Schizosaccharomyces pombe* MATPi¹⁴ proteins, showing ~35% identity with each. The *Kn1* homeodomain contains identical residues or similar substitutions in six of the eight positions most conserved among homeodomains, and contains the four invariant residues present in the putative recognition helix (helix 3) of all non-yeast homeodomains⁹. Secondary structure algorithms¹⁵ predict α helices for *Kn1* in the region of the helix-turn-helix motif and there are no helix-breaking residues in the predicted helical regions. In the *Kn1* gene, the homeobox is separated from amino-terminal coding sequences by the large (5 kb) third intron and is interrupted near the carboxy terminus of helix 1 (ref. 11) by intron four.

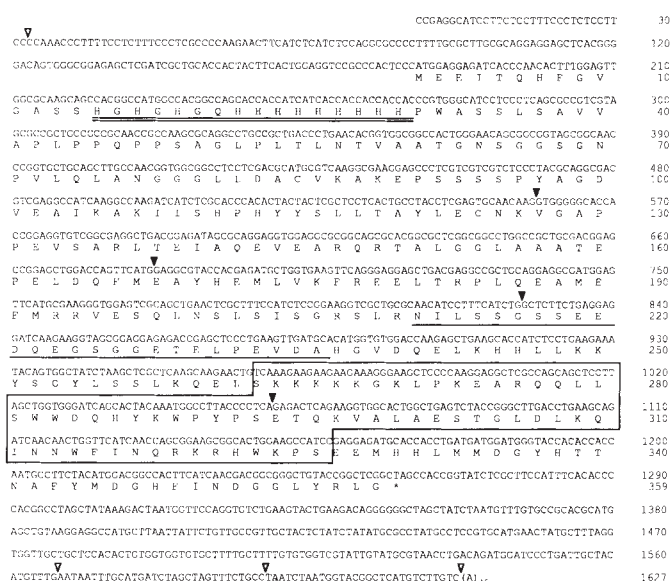


FIG. 1 Complementary DNA and predicted amino-acid sequence of maize *Kn1*. The first nucleotide in the sequence is the most 5' nucleotide identified by primer extension experiments (unpublished data); the open arrow at the 5' end indicates the 5' end of the longest cDNA; sequence upstream was derived from genomic clones. The amino-acid sequence of the open reading frame starts from the first ATG codon and is indicated beneath the nucleotide sequence. The histidine-rich or 'CAX' region, is doubly underlined and a candidate PEST sequence is singly underlined (see text). The homeodomain region is boxed. Filled arrows denote intron-exon junctions; the splice site donor and acceptor sequences were determined from genomic clones and agreed (not shown) with consensus sequences for plant introns³². The heterogeneity indicated at the 3' end of the transcript (open arrows) reflects different polyadenylation sites detected in the three sequenced cDNAs. Asterisk indicates the first in-frame stop codon.

METHODS. Complementary DNA primed with oligo(dT), was synthesized using poly(A)⁺ RNA from 14-day-old wild-type seedlings and cloned into the *EcoRI* site of λ gt10 (ref. 33). Six cDNA clones were isolated from a primary library of 500,000 recombinants using a probe derived from transposon-tagged genomic sequences⁵. The longest cDNAs were subcloned into Bluescript (Stratagene) plasmids. Double-stranded DNA was sequenced on both strands by the dideoxy-chain-termination method using T7 DNA polymerase and analysed with the PCGENE software package (Intelligenetics).

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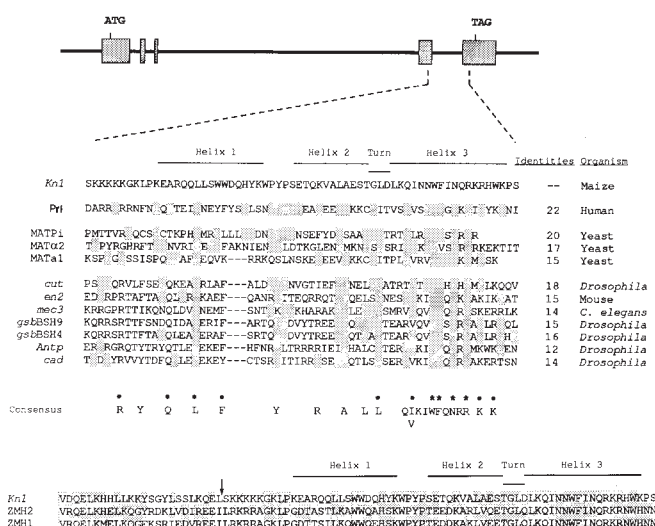


FIG. 2 Maize homeodomain sequences. *a*, The *Kn1* gene structure is above, dotted lines indicating the region that encodes the homeodomain. The schematic helix-turn-helix framework is positioned according to the corresponding motif of the Antp and engrailed homeodomains^{10,11}. The amino-acid sequence of several homeodomains is aligned with a segment of the *Kn1* sequence (residues 263 to 326). The three-residue gap (indicated by dashes) was introduced to maximize overall sequence similarity. Residues identical to those of *Kn1* are shaded. Asterisks identify the four residues invariant among multicellular eukaryotic homeodomains. The eight most highly conserved positions⁹ are indicated by dots. The consensus is derived from a collection of animal and fungal sequences⁹. *b*, An extended segment of the *Kn1* sequence (residue 239 to 326) aligned with portions of ZMH1 and ZMH2. Vertical arrow indicates the N terminus of the classical homeodomain used in panel *a*. Residues that are identical in at least two proteins are shaded.

METHODS. Sequence similarities were detected and aligned by either the FASTP programme⁸ or visual inspection. Amino-acid sequences in *a* other than *Kn1* and *Pr1*^{12,13} were taken from ref. 9. cDNAs encoding ZMH1 and ZMH2 were isolated from an amplified maize seedling cDNA library as described above for *Kn1*, with altered hybridization conditions³⁴.

Some *Drosophila* and *Caenorhabditis elegans* genes also contain introns in the homeobox¹⁶⁻¹⁹.

Outside the homeodomain, a few aspects of the *Kn1* amino-acid sequence are noteworthy. Near its amino terminus the sequence contains a short region rich in histidine (Fig. 1). Short polyhistidine blocks are present in only a few eukaryotic proteins, including the homeodomain proteins of *caudal* (*D. melanogaster*)²⁰ and *ERA-1* (mouse)²¹. No function has been assigned to these polyhistidine arrays. Many other developmentally important *Drosophila* proteins also contain short blocks of repeated amino acids, frequently resulting from repetition of the codon CAX^{16,20,22} (where X is any nucleotide) as in *Kn1*. From residue 211 to 236 (Fig. 1) there is a candidate PEST sequence. PEST sequences may act as signals for rapid degradation in proteins with short half-lives²³, suggesting that tight regulation of *Kn1* may be critical. Residue 128 to residue 166 shows 37% to 52% similarity to amphipathic α helix-forming regions of several mammalian apolipoproteins. Secondary structure calculations¹⁵ predict strong α -helical character for the corresponding *Kn1* segment, and helical wheel plots indicate amphipathic structures²⁴. Amino-acid sequences predicted to form amphipathic α helices can function in protein-protein interactions and as activation domains in other transcription factors^{25,26}. This portion of the *Kn1* protein, which comprises nearly all of exon 2, may define a module of similar function.

The *Kn1* homeobox was used as a hybridization probe to obtain homologous clones from a maize-seedling cDNA library. Two cDNAs were sequenced and the encoded proteins, which we have called ZMH1 and ZMH2 (*Zea mays* homeobox), contain homeodomains (Fig. 2*b*). ZMH1 and ZMH2 share identical residues in 57 of the 64 positions comprising the classical homeodomain, although they map to different chromosomal positions (B. Lowe and S.H., unpublished data); they share 35 residues with *Kn1* (55% identity), 15 of which are in the conserved 17-residue recognition helix. Sequence homology between the three maize proteins extends for 88 residues N-terminal of the homeodomain, with 48% identity. The four conserved residues at the N-terminal end of the similarity block (QELK in one-letter code; Fig. 2*b*) are reminiscent of the highly conserved sequence YPWM found upstream of many *Drosophila* homeodomains⁹, although adjacent residues are also conserved in the plant proteins. Outside the extended homeodomain, the three sequences differ. These data imply that a number of homeobox genes are expressed in maize, and that the *Kn1* homeobox should facilitate the identification and cloning of additional plant homeobox genes.

The presence of a homeodomain motif in the *Kn1* gene suggests that its gene product acts as a transcription factor. The

possibility that *Kn1* regulates genes involved in development is suggested by its mutant phenotype in which the timing and position of cell differentiation in the developing leaf are altered. In mutant plants, foci of cells along the lateral veins continue to divide and elongate into knots, whereas surrounding cells do not (Fig. 3*d*). The pattern of expression of photosynthetic proteins is altered in cells surrounding knots (N.S. and S.H., unpublished data). The ligule is often missing from its usual position (Fig. 3*b*), and/or found in ectopic patches in the leaf blade³. The differentiation of the ligule, veins and surrounding cells is highly position-dependent in normal leaf development^{27,28}. *Kn1*

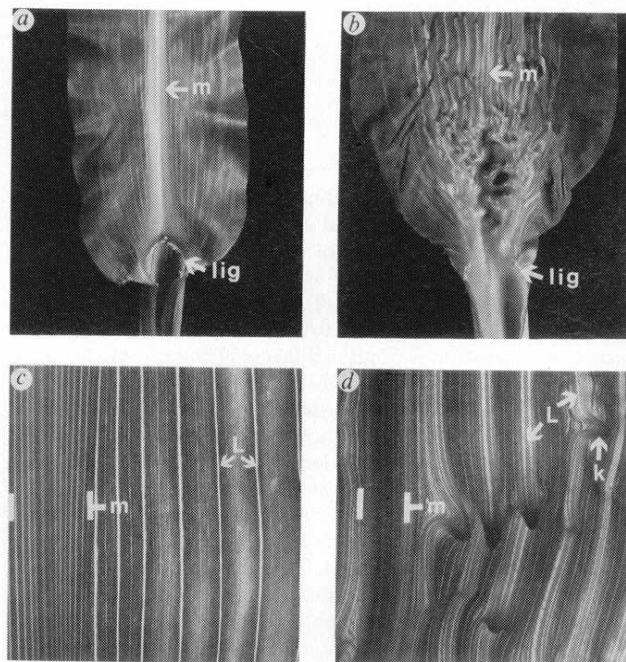


FIG. 3 The phenotype of normal and *Kn1-O* leaves. *a*, The eighth leaf from a wild-type plant. The ligule (lig) divides the sheath and blade. The midrib (m) runs through the middle of the leaf and is composed of closely appressed veins. *b*, The eighth leaf from a plant carrying the dominant *Kn1-O* allele. The ligule region is very distorted, sheath tissue and ligule fringe are displaced into the blade. *c*, Close-up of the wild-type leaf in *a*. Between the lateral veins (L) are 8-10 intermediate veins. *d*, Close-up of the *Kn1-O* leaf in *b*. Knots (k) (regions of extra cell divisions and elongation) occur sporadically along the lateral veins. The veins appear more prominent and have attributes of sheath (N.S. and S.H., submitted; P. Becraft and M. Freeling, personal communication).

mutations alter non-coding regions of the gene and do not substantially alter expression levels of the single *Kn1* transcript present in leaves (unpublished data). *Kn1* is expressed in embryos (data not shown) and a number of organs, but is more abundant in regions of increased mitotic activity (B. Greene and S.H., unpublished results). All mutations recovered thus far are dominant, which suggests that loss-of-function mutations may either have no phenotype or are lethal. A deletion that includes *Kn1* but not closely linked genes is lethal to the male gametophyte⁶ or when uncovered by a much larger deficiency, although the precise extent of the deletion has not been determined.

The *Kn1* homeodomain is equally similar to yeast and human sequences, consistent with their evolution from a common ancestral sequence before the divergence of the plant, animal and fungal kingdoms 10⁹ years ago. Given that this divergence occurred before the evolution of multicellularity, it is interesting that the *Kn1* gene, like many animal homeobox genes, appears to regulate processes that determine cell fate. Homeobox genes may regulate multicellular development using modifications of regulatory mechanisms that existed in unicellular ancestors. Alternatively, the ancestral homeobox gene may have had certain attributes which predisposed its descendants to independently acquire developmental functions.

Our data show that *Kn1* is a member of a larger class of plant-specific homeodomain genes. Sequence analysis reveals conserved sequences that extend beyond the motifs conserved in fungi and animals. By analogy with *Drosophila* homeobox genes^{20,29-31} that were cloned by homology and shown to have related developmental functions, we expect some members of the maize homeobox family to have functions similar to *Kn1*. Indeed, the *Kn1* homeobox has been used to clone homeobox-containing sequences that cosegregate with other leaf mutations (our unpublished data; and P. Becraft, S.H. and M. Freeling, unpublished data). Using the *Kn1* homeobox we are cloning related sequences from maize and other plants to investigate the functional and evolutionary significance of this plant-specific motif. □

Introduction of a subtle mutation into the *Hox-2.6* locus in embryonic stem cells

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GENE targeting in embryonic stem (ES) cells is a powerful tool for generating mice with null alleles¹. Current methods of gene inactivation in ES cells introduce a neomycin gene (*neo*) cassette both as a mutagen and a selection marker for transfected cells²⁻¹¹. Although null alleles are valuable, changes at the nucleotide level of a gene are very important for functional analysis. One gene family in which subtle mutations would be particularly valuable are the clusters of *Hox* homeobox genes¹²⁻¹⁶. Inactivation of genes in a cluster with a *neo* cassette that includes promoter/enhancer

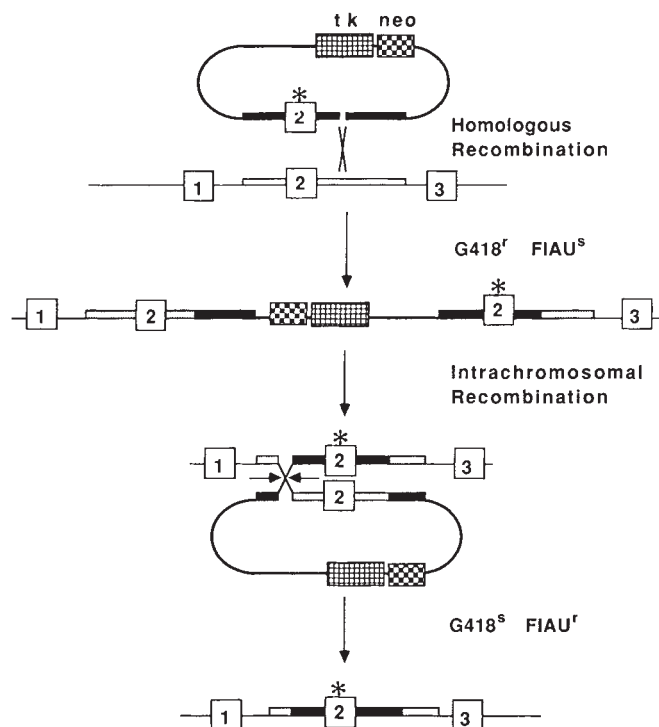


FIG. 1 Two-step recombination procedure used to introduce a small mutation into a nonselectable gene. The initial event involves single reciprocal recombination between the target gene and the insertion vector. The insertion vector contains a small mutation in the homologous DNA with the *neo* and *tk* genes located outside the region of homology. The vector is linearized within the region of homology at a unique restriction site. The entire vector integrates at the target gene and creates a duplication¹⁷⁻¹⁹ of genomic sequences. One, both or none of the duplicates may contain the desired mutation. The targeted cell becomes G418^r and FIAU^s. G418^r colonies are screened to find targeted events that contain the mutation. Intrachromosomal recombination subsequently occurs within the duplication²⁰. The plasmid, *neo*, *tk* and the duplication from either genome and/or vector origin are lost. The reverted cell becomes G418^s and FIAU^r and is screened for the presence of the mutation. Thick lines, homologous DNA; black, vector-derived; stippled, genomic-derived. Thin lines, nonhomologous genomic DNA. Lines of intermediate thickness, plasmid. Exons are boxed and numbered. *, The small mutation. Horizontal arrows, the plane of resolution within the Holliday structure²⁵.

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elements may deregulate transcription of neighbouring genes and generate a phenotype which is difficult to interpret. We describe here a highly efficient gene targeting method, termed the 'hit and run' procedure. This generates ES cells with subtle site-specific mutations with no selectable marker and may be useful for most genes. We have developed this procedure at the hypoxanthine phosphoribosyltransferase (*hprt*) locus and subsequently isolated ES cells with a premature stop codon in the homeobox of *Hox-2.6* (ref. 14).

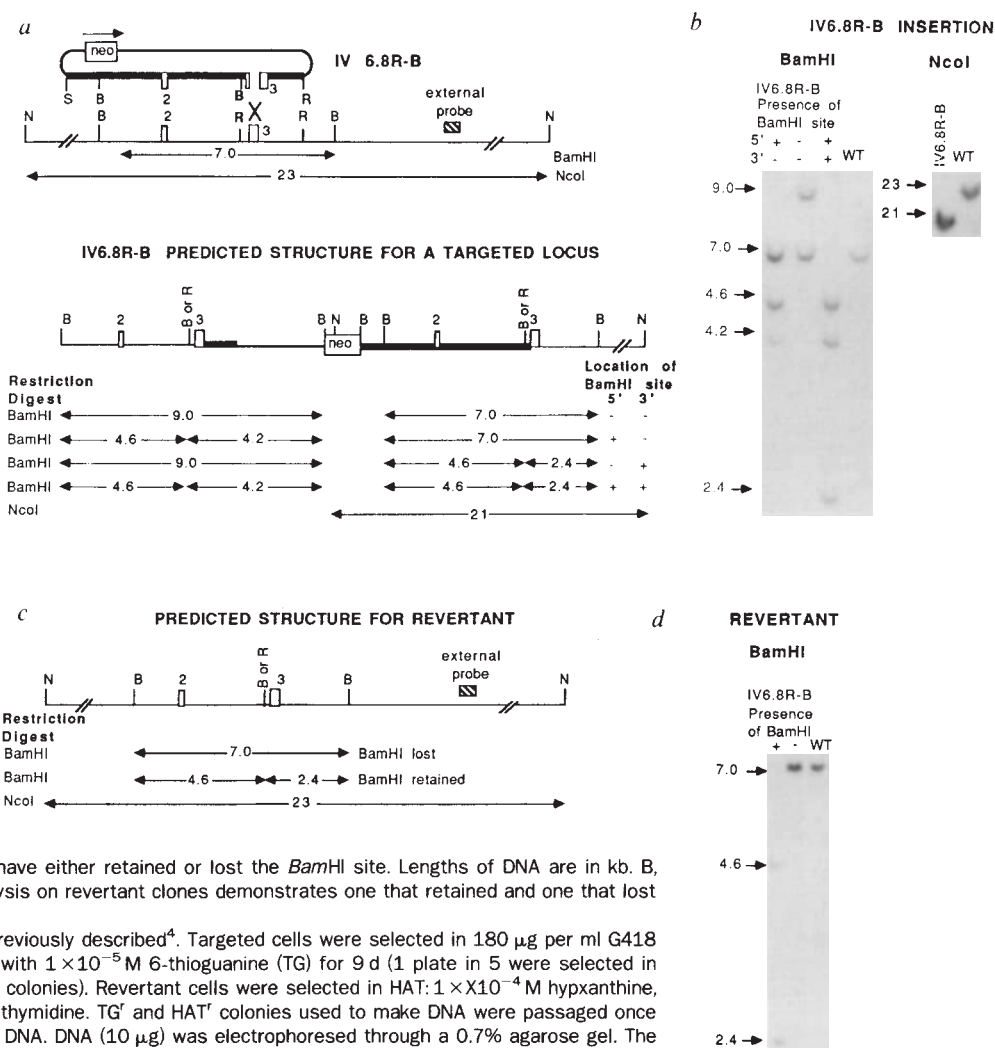
The hit and run procedure introduces a site-specific mutation into a nonselectable gene by a two-step recombination process (Fig. 1). The vector contains sequences homologous to the target gene, modified to include a desired mutation. The construct also has a *neo* gene and an HSV1-thymidine kinase (*tk*) gene in the plasmid backbone for positive and reversion selection respectively. In the first step, targeting occurs by single reciprocal recombination¹⁷⁻¹⁹ generating a duplication of homology separated by the plasmid and the selection cassettes. In the second step, the duplication can be resolved by single reciprocal intra-chromosomal recombination²⁰, which we refer to as reversion. The reversion removes *neo*, *tk*, the plasmid backbone and one copy of the duplicated homologous DNA. The resolution of the duplication will generate clones with the desired mutation and clones that are restored to wild type. The reversion event is selectable in FIAU²¹ for the loss of *tk*.

We tested the hit and run procedure with IV6.8R-B, an insertion vector⁸ with 6.8 kilobases (kb) of homology to the *hprt*²² locus. IV6.8R-B contains an oligonucleotide insertion that converts an *Eco*RI site to a *Bam*HI site in intron 2 (Fig. 2a). This vector was linearized inside the region of homology, electroporated into ES cells and selected in G418 and 6-thioguanine (TG) for targeted events. We recovered G418-resistant (G418^r) and TG-resistant (TG^r) colonies at a frequency of 1 per 123 G418^r colonies. Southern-blot analysis on 19 of these colonies confirmed that 17 were genuine targeted events and that the *Bam*HI mutation was present in the 5' duplicate for five clones, the 3' duplicate for one clone, both 5' and 3' duplicates for one clone and in neither duplicate for ten clones (Fig. 2a and b). This can be explained by gene conversion and heteroduplex repair during the initial insertion recombination event¹⁸.

The frequency with which the duplication resulting from the insertion is resolved may limit the applicability of the hit and run procedure. We measured the rate of reversion by the restoration of *hprt* function by survival in hypoxanthine-aminopterin-thymidine (HAT) media of a clone carrying the mutant *Bam*HI site in the 5' duplicate. The 6.8-kb duplication reverted at a rate of 4.3×10^{-6} per cell generation. Southern blot analysis on 15 HAT-resistant (HAT^r) colonies showed the duplication, *neo* and plasmid backbone were correctly excised from the *hprt* locus and two revertants retained the *Bam*HI mutation (Fig. 2c and

FIG. 2 Insertion and reversion at the *hprt* locus. a, IV6.8R-B is a 6.8-kb *Sac*I-*Eco*RI *hprt* fragment that contains exons 2 and 3 which was cloned into the *Sac*I and *Eco*RI sites of pTZ19r (Pharmacia). MC1neopA⁸ was inserted into the polylinker. IV6.8R-B was linearized at the *Xho*I site in exon 3. A 27-base pair (bp) oligonucleotide, 5'-AA-TTGCCGGATCCGGCCGGATCCGGGC-3' was inserted into an internal *Eco*RI site (destroyed) to make a *Bam*HI site. Predicted fragments are illustrated for a *Bam*HI digest with the mutant *Bam*HI site in either the 5', 3', neither or both duplicates. Probes for Southern analysis are: an internal probe (*hprt* exons 2 and 3 from cDNA cut with *Hinc*II and *Hpa*I²²) and a 3' external probe (a 0.5-kb fragment cut with *Pst*I and *Hind*III just 5' to exon 4) represented by a rectangle filled in with diagonal lines. Thick lines, vector *hprt*; thin lines, genomic *hprt*; lines of intermediate thickness, plasmid. Lengths of DNA are in kb. B, *Bam*HI; R, *Eco*RI; N, *Nco*I; S, *Sac*I. b, Southern analysis of TG^r clones with *Bam*HI and *Nco*I digests. Various *Bam*HI restriction patterns are shown hybridized to the internal probe. The *Nco*I restriction pattern, hybridized to the external probe, was the same for all *Bam*HI restriction patterns, therefore only one clone is shown. WT, wild type. c, Illustration of the predicted structure for revertant HAT^r clones that have either retained or lost the *Bam*HI site. Lengths of DNA are in kb. B, *Bam*HI; R, *Eco*RI; N, *Nco*I. d, Southern analysis on revertant clones demonstrates one that retained and one that lost the *Bam*HI mutation. WT, wild type.

METHODS. Electroporation was done as previously described⁴. Targeted cells were selected in 180 μ g per ml G418 for 5 d and then in 180 μ g per ml G418 with 1×10^{-5} M 6-thioguanine (TG) for 9 d (1 plate in 5 were selected in G418 only to obtain the number of G418^r colonies). Revertant cells were selected in HAT: 1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterin and 1.6×10^{-5} M thymidine. TG^r and HAT^r colonies used to make DNA were passaged once without feeder cells to reduce feeder cell DNA. DNA (10 μ g) was electrophoresed through a 0.7% agarose gel. The *Nco*I-digested DNA was run with field inversion gel electrophoresis optimized to separate 15–25 kb fragments. DNA was transferred onto a Genescreen Plus filter (Dupont) and hybridized to the specified probe under standard conditions.



d). Therefore, insertion and reversion occur at a high frequency with 6.8 kb of *hprt* homology and revertants retaining the mutation can be recovered at this locus.

This suggested that the hit and run procedure may be applicable to genes in which the recombination events are not directly selectable. To test this we introduced a specific small mutation that creates a diagnostic *NheI* site and stop codons into the *Hox-2.6* homeobox²³ (Fig. 3a). The translated product should be 43 amino acids shorter than the normal protein.

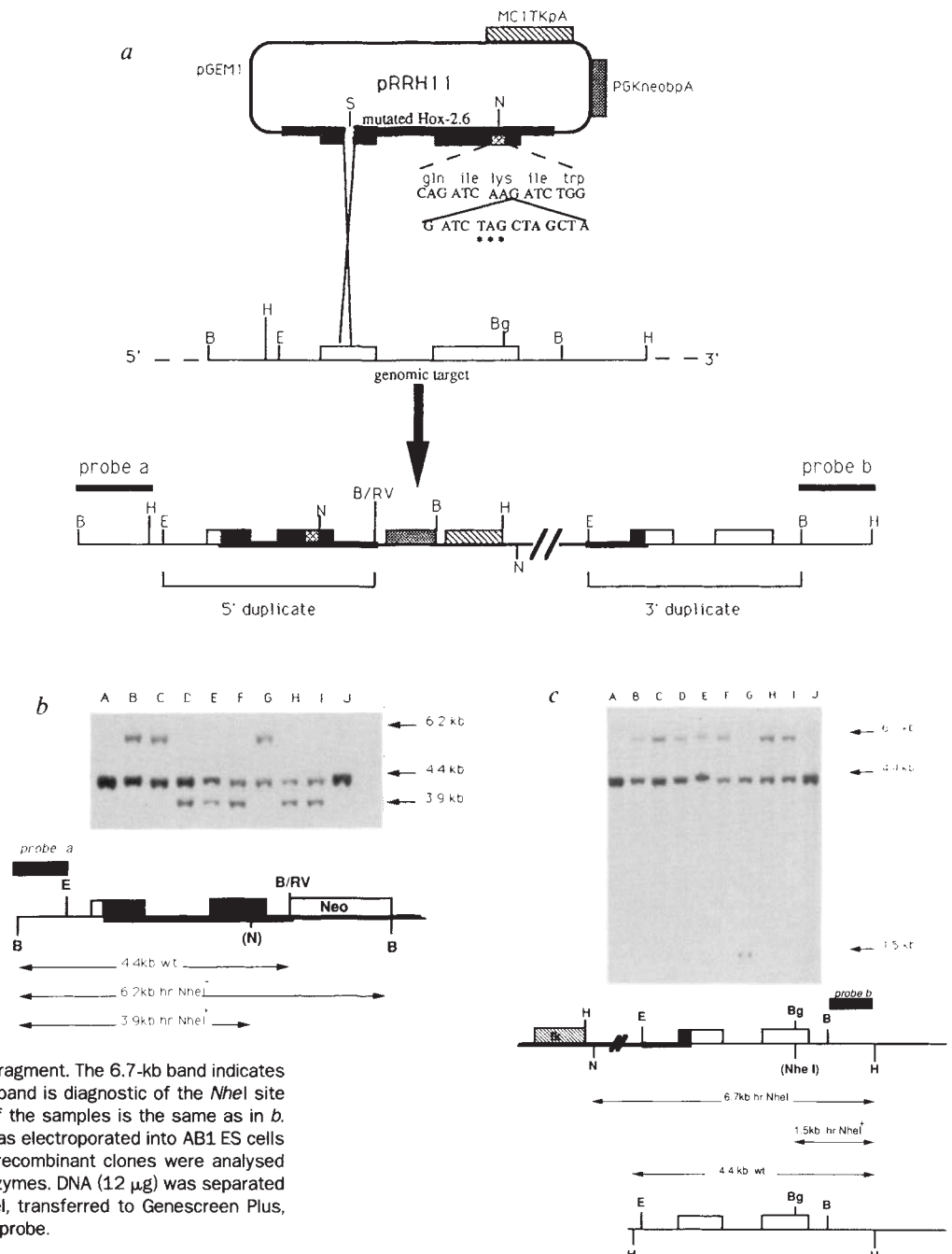
For the insertion event, the vector was linearized in the region of homology. G418^r ES cell clones were pooled and screened by Southern analysis; targeted events were detected at a frequency of 1 to 31 homologous to nonhomologous integrations. Cells from four positive pools were plated at low density to isolate homologous recombinant clones. Two positive clones

from each pool were selected for detailed analysis of the presence and position of the *NheI* mutation (Fig. 3b and c).

The presence of the mutation was verified by Southern analysis with double digests using flanking probes specific for the 5' and 3' elements of the duplication (Fig. 3). Out of eight clones analysed, two had lost the *NheI* site (Fig. 3b and c: clones B, C), five had the *NheI* mutation in the 5' duplicate (clones D, E, F, H, I) and one had the *NheI* mutation in the 3' duplicate (clone G). These patterns can also be explained by gene conversion and heteroduplex repair.

To measure the rate of reversion, one targeted clone was tested for the loss of *tk* activity by selection in FIAU (Fig. 4a). We obtained FIAU^r colonies at a rate of 3.8×10^{-3} per cell generation. This was 1,000 times greater than the reversion frequency observed for *hprt* and it could be that *Hox-2.6* is

FIG. 3 Targeted insertion of pRRH11 into the *Hox-2.6* locus. a, pRRH11 is an insertion vector that contains a 3.1-kb *Hox-2.6* fragment. This includes the two exons of the gene that have been modified by the introduction of the oligonucleotide 5'-GATCTAGCTAGCTA-3' into the *Bgl*II site to generate a stop codon and a new *NheI* site. Downstream of the *Hox-2.6* gene is a PGKneobpA expression cassette (a *neo* gene transcribed from a mouse phosphoglycerate kinase promoter²⁶ with the bovine growth hormone gene polyadenylation signal²⁷) and an MC1tkpA cassette (an HSV1-*tk* gene driven by the MC1 promoter-enhancer unit⁸). The vector was linearized at the *Sal*I site in exon 1. It integrates creating a duplication of the *Hox-2.6* gene interrupted by the PGKneobpA, MC1tkpA, and pGEM-1 sequences. B, *Bam*HI; H, *Hind*III; E, *Eco*RI; N, *Nhe*I; Bg, *Bgl*II; RV, *Eco*RV; S, *Sal*I; *** stop codon. Probe a is the 1.2-kb *Bam*HI-*Eco*RI fragment and probe b is the 1.0-kb *Bam*HI-*Hind*III fragment; both probes are absent from the targeting vector. b, To examine the structure of the 5' duplicate in homologous recombinant clones, a *Bam*HI-*Nhe*I double digest was hybridized with probe a. Lanes A and J are negative controls. Lanes B-I, represent targeted clones. The 4.4-kb band is the wild-type allele; the 6.2-kb band indicates the presence of the vector but absence of the *NheI* site in the 5' duplicate and the 3.9-kb band indicates the presence of the *NheI* site in the 5' duplicate. Intensity variation of the mutant allele with respect to the wild type was due to feeder cell DNA. c, To examine the structure of the 3' duplicate, a *Hind*III-*Nhe*I double digest was hybridized with probe b.



The 4.4-kb band is the wild type *Hind*III fragment. The 6.7-kb band indicates the presence of the vector. The 1.5-kb band is diagnostic of the *NheI* site present in the 3' duplicate. The order of the samples is the same as in b. METHODS. Linearized pRRH11 (20 μ g) was electroporated into AB1 ES cells as previously described⁴. Homologous recombinant clones were analysed by a double digest with the indicated enzymes. DNA (12 μ g) was separated by electrophoresis in a 1% agarose gel, transferred to Genescreen Plus, and hybridized to the indicated labelled probe.

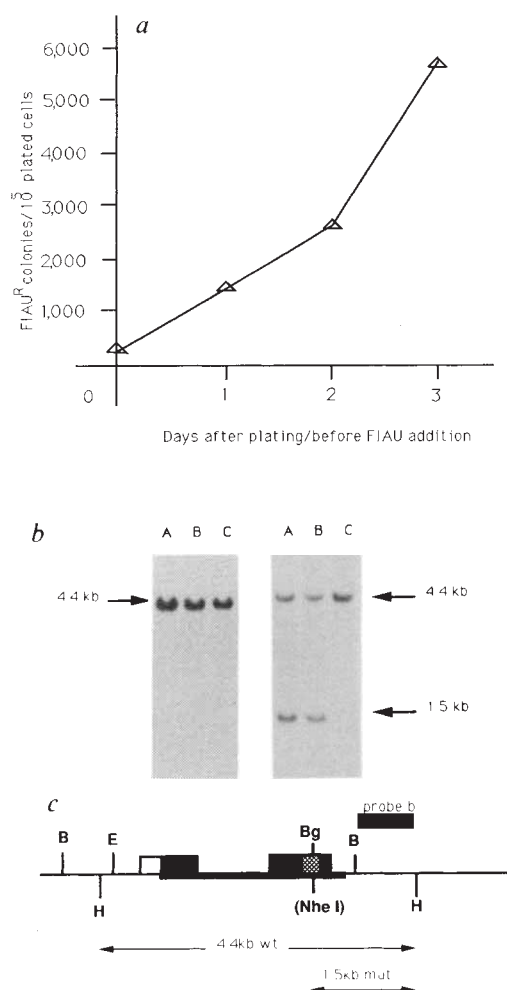


FIG. 4 Reversion at the *Hox-2.6* locus. **a**, Frequency of reversion at the *Hox-2.6* locus. Cells were grown in the presence of G418 for 10 d to select against the expansion of an early reversion event and then plated at a density of 1×10^5 cells per 90-mm plate. G418 was removed and the cells were selected for the loss of *tk* in 0.2 μ M FIAU. FIAU was added to duplicate plates on day zero, then every 24 h for 3 d. The reversion rate was calculated by averaging the reversion frequencies between days 1–2 and days 2–3. The frequency was calculated as follows: (number of colonies on day X)/(number of colonies on day X-1)/(total number of cells on day X). Cell generation time was determined to be 1.05 cell generations per 24 h. **b**, Southern analysis of revertant clones. *Hind*III-digest of genomic DNA from FIAU^r clones hybridized with probe b shows a single 4.4-kb wild-type band indicative of the absence of the vector. **c**, Identification of clones with the *Nhe*I mutation. *Hind*III-*Nhe*I double digest of the same samples in **4b**, hybridized with probe b shows the 4.4-kb band that corresponds to the wild-type allele and the 1.5-kb band that corresponds to the mutated allele. Clones A and B have the mutation, clone C reverted back to wild type. **METHODS**. Genomic DNA was prepared from FIAU^r clones, digested, separated in a 0.8% agarose gel by electrophoresis, transferred to GeneScreen Plus and hybridized to the indicated labelled probe.

much more prone to recombination. FIAU^r colonies were screened by Southern analysis for the excision of the vector and the presence of the *Nhe*I mutation. Revertant colonies from two independent insertion events have excised the duplication and retained the *Nhe*I mutation in the *Hox-2.6* gene (Fig. 4b and c, clones A and B).

We have used the hit and run procedure to introduce small mutations in the *hprt* and *Hox-2.6* genes without a selectable marker. This is particularly important in the *Hox-2* cluster as there are many genes located close together and the insertion of a promoter/enhancer associated with the *neo* gene may affect

multiple coding units. The specific termination codon in the *Hox-2.6* homeobox will provide a clear interpretation of a potential phenotype when this allele is established in the germ line. Microinjection²⁴ coupled with direct screening is a technically demanding alternative approach to generate small site-specific mutants. However, the hit and run procedure can be used to make very specific mutations in genes using conventional transfection and selection technologies. This will facilitate detailed *in vivo* studies of protein structure and function not previously possible with the insertion of a selectable marker. □

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Coordination of expression of DNA synthesis genes in budding yeast by a cell-cycle regulated *trans* factor

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ALL of the DNA synthesis genes of budding yeast examined so far are periodically expressed and hence under cell-cycle control (Table 1). Expression occurs near the G1/S phase boundary¹⁻³ and the genes seem to be coordinately regulated (reviewed in ref. 4). The upstream promoter sequences of these genes have only a hexamer element, ACGCGT (an *Mlu*I restriction site), in common. Here we show that this hexamer is able to impart periodic expression to a heterologous gene and, significantly, this expression occurs coincidentally with that of *CDC9*, one of the DNA synthesis genes (Table 1). We have also identified a protein that binds specifically to these sequences in a similar periodic manner. These ACGCGT sequences and the transcription factor that binds to them therefore seem to be the elements controlling both the periodic expression and coordinate regulation of the DNA synthesis genes.

Apart from *CDC21* and *POL1*, most of the DNA synthesis genes have only a single ACGCGT sequence. But some (for example, *CDC9*; see below) have additional related sequences in which the CGCG core is conserved. We inserted synthetic copies of the ACGCGT hexamer into the high copy number vector pLGΔ178 (ref. 5), which contains a minimal promoter from the yeast cytochrome *c* gene fused to the *Escherichia coli lacZ* gene. A single ACGCGT sequence in this vector produced negligible β -galactosidase activity. However, the presence of two or three ACGCGT hexamers resulted in a very large stimulation of β -galactosidase (Table 2). In contrast, up to seven copies of a mutated version of the hexamer, ActaGT, had a negligible effect on β -galactosidase activity (Table 2). Hence the ACGCGT hexamer sequence formally acts as an upstream activation sequence element, and the nature of this activation may be explained by the synergistic interaction of multiple activation sites^{6,7} or by cooperative binding of a protein to the repeated elements. Significantly, the cell-cycle regulated *HO* and histone

TABLE 2 Stimulation of β -galactosidase expression by the ACGCGT sequences

Construct	β -galactosidase level	Construct	β -galactosidase level
pLGΔ178	0.02	1 × ActaGT	0.03
1 × ACGCGT	0.03	3 × ActaGT	0.03
2 × ACGCGT	0.47	5 × ActaGT	0.03
3 × ACGCGT	2.55	7 × ActaGT	0.03

Synthetic copies of the ACGCGT hexamer and a mutated version of it, ActaGT, were inserted into the *Xho*I restriction site of vector pLGΔ178 (see text). The constructs shown above (confirmed by DNA sequencing) were transformed into the strain CG378 (ref. 23) and assayed for β -galactosidase. The results are expressed as absorbance at 420 nm per hour per 50 μ g crude cell protein and each is the average of multiple assays performed on at least three independent transformants.

H2A-H2B genes are also driven by short, but different, sequence elements⁸⁻¹⁰.

A yeast strain containing the plasmid pLGΔ178.3M, which carries three of the hexamer elements controlling *lacZ* expression, was synchronized using the yeast mating pheromone α -factor (Fig. 1a). The sharp periodicity of the *CDC9* and histone H2A transcripts shows that the resultant culture was highly synchronous over two to three cycles. The *lacZ* transcript, instead of declining during the 4-hour incubation in α -factor, as occurred with *CDC9* and histone H2A, increased two- to threefold and then was not expressed in the first cycle after release. However, periodicity of the *lacZ* transcript is evident in the second and third cycles and, importantly, it is coincident with *CDC9*.

Induction methods of synchrony may give rise to artefacts, and we therefore also prepared a synchronous culture by elutriation. Synchrony with this system is usually poorer than with α -factor release, although the periodic histone H2A and *CDC9* mRNAs indicate reasonable synchrony over two cell cycles (Fig. 1b). However, expression of *lacZ* again preceded *CDC9* in the first cycle. Indeed, only the second half of the peak is evident. In the second cycle *lacZ* expression is clearly periodic and, as with α -factor synchrony, it is in phase with *CDC9* transcription. Thus the ACGCGT hexamers, by themselves, are able to impart periodic transcription to the heterologous *lacZ* gene at the correct point in the cell cycle. However, this expression is clearly extremely sensitive to physiological perturbation.

The promoter region of the *CDC9* gene contains one ACGCGT hexamer centred at position -122 relative to the ATG translational initiation codon, and one near-match, ACGCGa, centred at position -130 (ref. 11). When these were deleted from a plasmid in which the *CDC9* promoter region was fused to *lacZ*, periodicity of *lacZ* transcription was abolished (Fig. 1c). The deletion, which extends from position -162 to -102, removes other surrounding sequences as well, but it supports the notion that it is the ACGCGT hexamer elements, and perhaps near matches to the consensus, that are important in regulating periodic transcription at the G1/S phase boundary.

We have used gel retardation assays^{12,13} to identify the factor that binds to the ACGCGT sequences (Fig. 2a). As substrates we tested fragments containing either one, two or three ACGCGT repeats. All three showed several retarded bands (Fig. 2a; lanes 2, 6 and 10) but of these, only the uppermost is specific as it is competed away in the presence of multimers of the ACGCGT hexamer (lanes 7, 8, 19, 20). Moreover, this upper band could not be detected with a substrate containing only one ACGCGT sequence, was only weakly detectable with two ACGCGT repeats but was clearly evident with three ACGCGT repeats. Thus, these data precisely reflect the transcriptional activity of these sequences (Table 2). Furthermore, the relative

TABLE 1 DNA synthesis genes that are periodically expressed in the cell cycle

Gene	Gene product	Reference
<i>CDC21</i>	Thymidylate synthase	18
<i>CDC9</i>	DNA ligase	1
<i>CDC8</i>	Thymidylate kinase	2
<i>POL1</i>	DNA polymerase I	3
<i>POL2</i>	DNA polymerase II	*
<i>DPB2</i>	DNA polymerase II subunit B	*
<i>DPC2</i>	DNA polymerase II subunit C	*
<i>POL3 (CDC2)</i>	DNA polymerase III	19
<i>POL30</i>	PCNA	19
<i>PRI1</i>	DNA primase I	20
<i>PRI2</i>	DNA primase II	21
<i>RNR1</i>	Regulatory subunit of ribonucleotide reductase	22

* Our unpublished observations (with A. Sugino, National Institute of Environmental Health Sciences, North Carolina).

binding efficiency of these substrates is reflected in their efficiency as competitor DNA fragments (Fig. 2a, lanes 11–16). Further emphasizing the specific retardation of the upper band, competition did not occur with the mutated version of the *Mlu*I hexamer described above (ACtAGT; Fig. 2a, lanes 25–26). Finally, competition did occur with a 55-base pair restriction

fragment from the *CDC9* promoter region that corresponds almost exactly to the 60-base pair deletion used in Fig. 1c (Fig. 2a, lanes 17, 18). This fragment contained the single *CDC9* *Mlu*I site, showing that in the correct context the binding activity recognizes a single ACGCGT (although this fragment also contains a near match to the consensus) and it confirms that

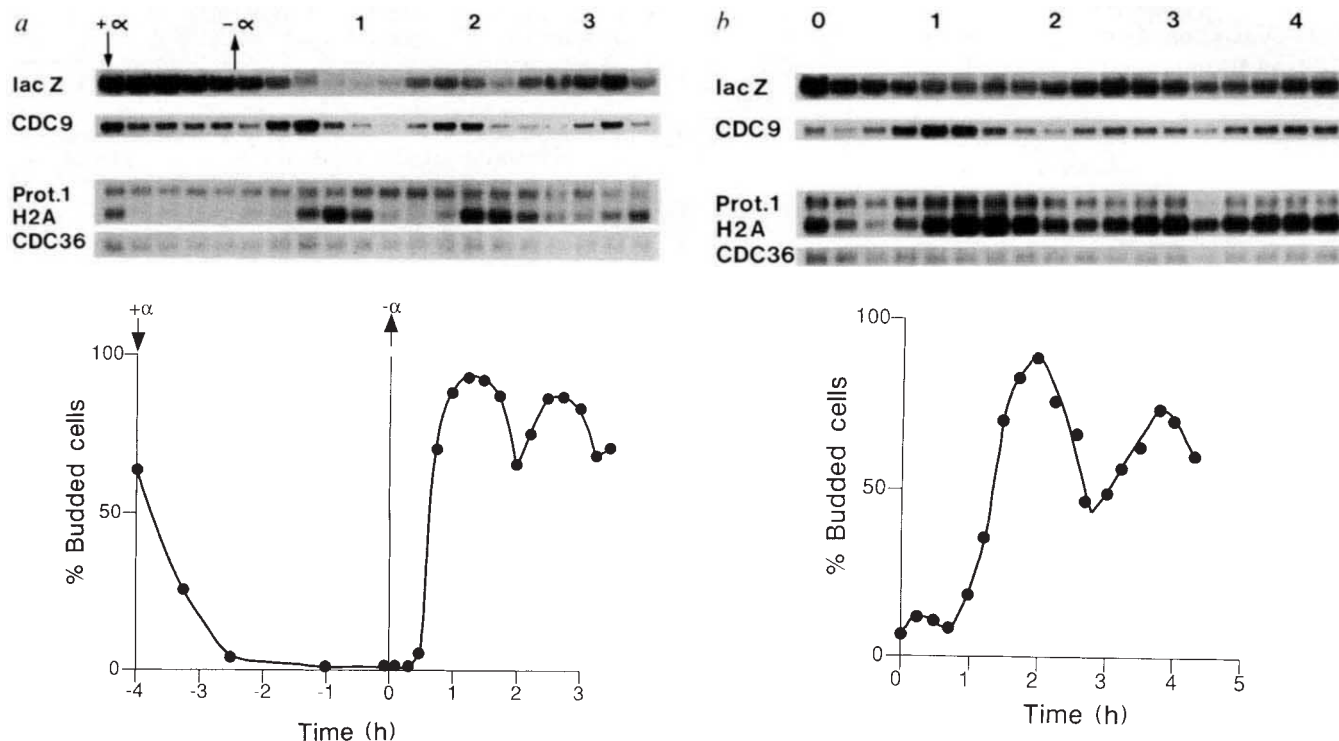
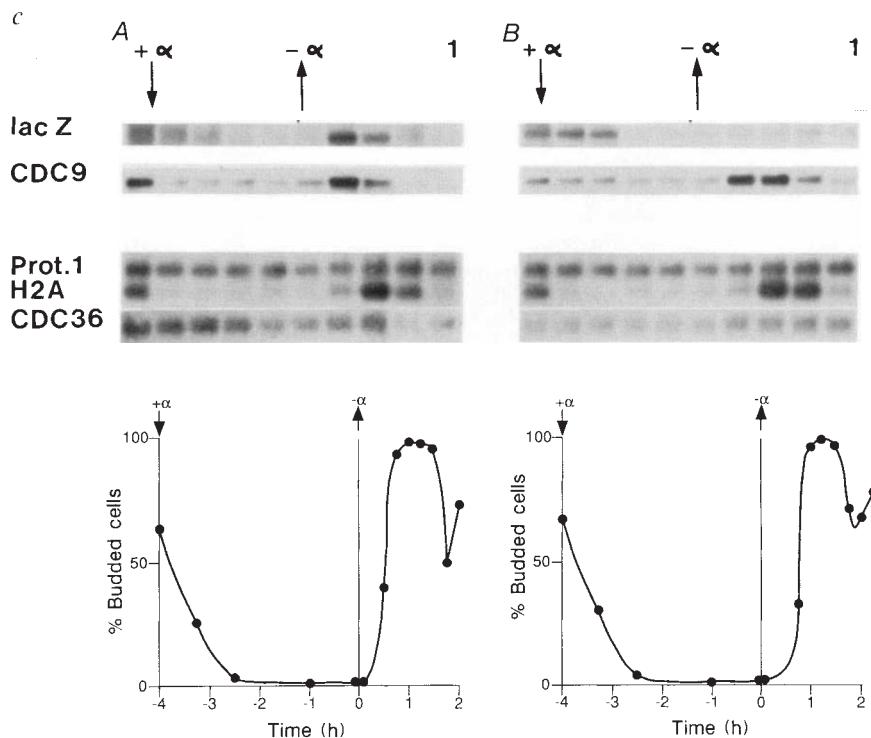


FIG. 1 Cell-cycle regulation of the *lacZ* transcript under control of the ACGCGT sequences in synchronized cultures and the effect of deleting the ACGCGT sequences from the *CDC9* promoter region. a, Northern blot analysis of RNA from cells synchronized by α -pheromone arrest and release and b, by elutriation. c, Northern blot analysis was carried out on cells synchronized by α -pheromone containing A, the intact *CDC9* promoter fused to the *lacZ* gene²³ and B, a deletion of the ACGCGT sequences from this promoter (see text). For each experiment the degree of synchrony obtained is shown by plotting the percentage of budded cells against time. The figures above the data points are times in hours. The levels of protein 1 and *CDC36* transcripts act as loading controls as they are invariant in the cell cycle.

METHODS. a, A culture of strain CG378 (ref. 23) containing pLGΔ178.3M was grown to mid-log phase in YNB³. After sampling, α -factor was added at 2.5 $\mu\text{g ml}^{-1}$ and further sampling was at 45 min, 90 min, 3 h and 4 h. The α -factor was then removed and after resuspension at 5×10^6 cells ml^{-1} , further samples were taken at 0 min and then at 15-min intervals. b, A mid-log phase culture of a diploid of CG378 and YNN27²⁰ containing pLGΔ178.3M was loaded at a rate of 120 ml per min into a 40 ml chamber in a Beckman JE-5.0 elutriator rotor running at 4,500 r.p.m. A population of small, unbudded cells was then eluted at a flow rate of 150 ml per min and a culture was set up at 2×10^6 cells per ml. Samples were removed at 15-min intervals. c, The *CDC9* promoter, fused to the *lacZ* gene²³, was treated with the nuclease *Bal*/31 to introduce appropriate deletions²⁴. Strain CG378 containing plasmid pEMBLye23 (ref. 25) carrying either A, the intact *CDC9* promoter fused to *lacZ* as a control or B, the required promoter deletion (see text) fused to



lacZ, was synchronized using α -pheromone (see above). During incubation in α -factor, samples were removed at the intervals stated in a and then at 0 min and at 15-min intervals after its removal. For these experiments, only the first 10 samples from each of A and B were analysed. RNA extraction, northern blotting and probing have been described¹.

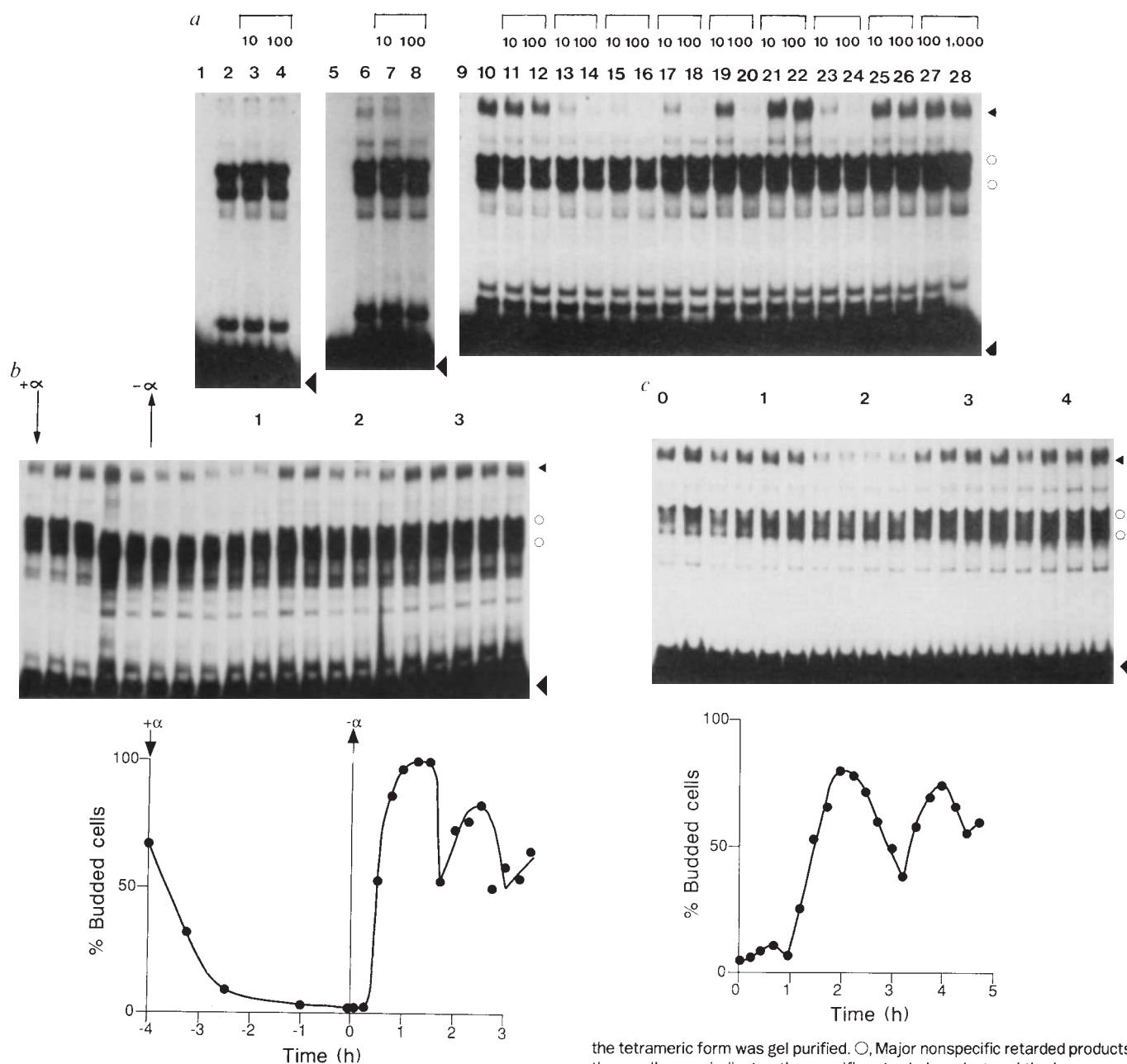


FIG. 2 Gel retardation analysis showing *a*, binding of a specific protein to the ACGCGT sequence element, and *b*, binding of this protein during the cell cycle in an α -factor synchronized culture and *c*, cell cycle binding in a culture synchronized by elutriation. *a*, Clarified crude extracts²⁶ prepared from strain CG378 were used with the following probes (isolated from pLGΔ178 (ref. 5) derived constructs): lanes 1–4, the 49-bp *AluI/Sau3A* fragment from pLGΔ178.1M which contains one ACGCGT oligonucleotide; lanes 5–8, the 61-bp *AluI/Sau3A* fragment from pLGΔ178.2M containing two ACGCGT repeats and lanes 9–26, the 73-bp *AluI/Sau3A* fragment from pLGΔ178.3M containing three ACGCGT repeats. The competitor DNAs used were as follows (the molar excess in each case is shown above the appropriate lane): lanes 1, 5 and 9, unbound probe; lanes 2, 6 and 10, no specific competitor; lanes 3–4, 7–8 and 19–20, multimeric form of the ACGCGT oligonucleotide, 5'-TCGAGACGCGTC-3'; lanes 11–12, the 41-bp *AluI/Sau3A* fragment; lanes 13–14, the 61-bp *AluI/Sau3A* fragment; lanes 15–16, the 73-bp *AluI/Sau3A* fragment; lanes 17–18, a 55-bp *MluI/AluI* fragment from the *CDC9* promoter region mapping between positions –160 to –105 relative to the ATG codon; lanes 19–20, multimeric form of the ACGCGT oligonucleotide; lanes 21–22, multimeric form of an oligonucleotide containing an unrelated hexamer, 5'-TCGAGATGATTC-3'; lanes 23–24, tetrameric form of the *MluI* site oligonucleotide; lanes 25–26, tetrameric form of the mutated *MluI* site oligonucleotide, 5'-TCGAGACtGTC-3'; lanes 27–28, monomeric form of the *MluI* site oligonucleotide. The multimeric forms of the competitor oligonucleotides were generated by ligation and either total ligation products were used or

the tetrameric form was gel purified. ○, Major nonspecific retarded products; the small arrow indicates the specific retarded product and the large arrow indicates the unbound probe. *b*, Clarified crude extracts²⁶ were prepared using samples taken from a culture synchronized by α -factor release (see Fig. 1*a*) and used in a gel retardation assay with the 73 base pair (bp) *AluI/Sau3A* probe from pLGΔ178.3M. Note that lane 4 is overloaded and that the experiment has been confirmed by repetition using an independently synchronized culture of the same strain. *c*, Clarified crude extracts were prepared using samples taken from a culture synchronized by elutriation (see Fig. 1*b*) and again the 73-bp *AluI/Sau3A* fragment was used as a probe. Note that because of the large number of cells required for both RNA and protein preparations we are not currently able to prepare both RNA and protein from the same elutriated culture.

METHODS. All binding reactions were performed with 1 ng [γ -³²P] 5' end-labelled *AluI/Sau3A* restriction fragments at $\sim 1 \times 10^8$ c.p.m. μg^{-1} . *a*, Clarified crude extracts were prepared from strain CG378 grown to mid-log in YEPD medium. *b*, CG378/pLGΔ178.3M grown in YNB and synchronized with α -factor. *c*, YNN27 (ref. 20) $\times$ CG378/pLGΔ178.3M diploids synchronized by elutriation. Protein was estimated according to ref. 27 and 20 μg of protein were used per reaction. Binding buffer was: 50 mM Tris-HCl (pH 7.5); 50 mM KCl; 25 mM MgCl₂; 25% glycerol; 1 mM DTT; 0.1 mM PMSF; 5 $\mu\text{g ml}^{-1}$ chymostatin; 5 $\mu\text{g ml}^{-1}$ leupeptin; 5 $\mu\text{g ml}^{-1}$ antipain; 1 $\mu\text{g ml}^{-1}$ pepstatin; 0.1 $\mu\text{g ml}^{-1}$ poly(dI-dC).poly (dI-dC). The resulting complexes were fractionated by electrophoresis through non-denaturing 5% (30:1) polyacrylamide gels and run in 0.5% TBE. Gels were dried on Whatmann 3MM paper and autoradiographed.

we are dealing with a factor that can interact with sequences from the intact *CDC9* promoter. We have named this factor DSC1 (for DNA synthesis control).

When DSC1 activity was examined in an α -pheromone synchronized culture, it was found to mirror the expression of *lacZ* (Fig. 2b, and see Fig. 1a). The binding activity in the extracts increases during the α -factor incubation, then declines during the first cell cycle. The peak in binding from 75 min to 90 min corresponds to the second cycle peaks in the *lacZ* and *CDC9* transcripts, although it occurred somewhat earlier, as also happened in the third cell cycle. The binding appears weak relative to the strong transcript signal, but this is to be expected given the nature of DNA-protein interactions and given that each interaction is likely to stimulate many transcriptional initiation events. The periodic binding of DSC1 is also evident in a culture synchronized by elutriation (Fig. 2c). The pattern of binding is once more similar to the pattern of *lacZ* expression (Fig. 1b), although it is not identical. This may simply reflect the fact that these experiments were, of necessity, performed with different synchronous cultures (see legend to Fig. 2). Furthermore, synchrony decays rapidly between the second and third cycles in elutriated cultures. Nonetheless, this result, together with the result from the α -pheromone synchronized culture (Fig. 2b), supports the hypothesis that the binding of DSC1 to the ACGCGT hexamers is responsible for the periodic expression of *lacZ* and, by inference, of the DNA synthesis genes.

Both methods of synchrony give initial high levels of *lacZ* expression and DSC1 binding that clearly precede *CDC9* expression. Possibly, a transcription complex is formed on the endogenous *CDC9* promoter when DSC1 is present and this persists to give subsequent, correctly timed, expression of *CDC9*. This suggests that although the *MluI* motifs and DSC1 appear to be responsible for both the periodic and coordinate expression of the DNA synthesis genes listed in Table 1, they are only the core of a more complex system. It may be significant that in the *HO* and the histone H2A-H2B genes there are also negative regulators that control the activity of the cell cycle regulatory elements^{14,15}. A similar negative element may prevent the premature expression of *CDC9*, like that observed with *lacZ* when the ACGCGT sequences are located out of context on pLGΔ178. Moreover, some of the DNA synthesis genes contain only a single ACGCGT sequence which our data suggest is not active in isolation. Other proteins and sequences must therefore be involved in enhancing the activity of a single hexamer in endogenous promoters. Consistent with this, *MluI* sites also occur in the promoter regions of non-periodic genes.

To our knowledge, DSC1 is the only transcription factor periodically active in the cell cycle which has been described to date. The only other similar factor binds upstream of the hamster histone H3 gene, but this factor has been characterized only in serum-stimulated cells¹⁶. Furthermore, the activity recognizing the CACGAAA repeats of the *HO* gene seems to remain bound throughout the cell cycle¹⁷. The binding of DSC1 relative to the periodic expression of the *lacZ* and *CDC9* messages in the second and third cycles of the α -pheromone synchronized culture argues that DSC1 is normally produced (and/or activated) near to the G1/S phase boundary when it is required and degraded (and/or deactivated) shortly afterwards. This suggests it is likely to be one of the key proteins controlling DNA synthesis and therefore its regulation may be crucial to understanding the cell-cycle control of S phase. □

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Generating yeast transcriptional activators containing no yeast protein sequences

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WE previously reported that roughly 1% of the short peptides encoded by *Escherichia coli* genomic DNA fragments act as transcriptional activating regions in yeast when fused to GAL4(1-147), a DNA-binding portion of the yeast transcriptional activator GAL4 (ref. 1). Struhl questioned the conclusion that we had identified new transcriptional activating sequences that function in the absence of yeast transcriptional activating sequences². His criticism was based on two considerations: first, GAL4(1-147) contains an acidic segment (and subsequent experiments have shown that this region contains a weak activating region *in vitro*³); second, attempts to isolate new activating regions failed when the DNA-binding domain of a bacterial repressor, LexA(1-87), was used as the DNA-binding unit². We report here a repeat of our original experiment using the complete LexA molecule LexA(1-202) as the DNA-binding region, instead of GAL4(1-147) or LexA(1-87). We find that, as in the original experiment, about 1% of the short peptides encoded by *E. coli* genomic fragments act as transcriptional activating regions when fused to intact LexA. All of the new activating regions whose sequences we determined bore an excess of acidic amino acids (see Table 1).

We suggest that the relevant difference between LexA(1-202) and LexA(1-87) is that the former, like GAL4(1-147) (ref. 4), efficiently forms dimers—the DNA-binding species—but the latter does not⁵. It is also possible that the carboxyl domain of LexA helps to stabilize the proteins in yeast. Consistent with these ideas, we find that of five activating regions isolated as fusions to GAL4(1-147), all activated transcription when transferred to LexA(1-202), but only one activated transcription when attached to LexA(1-87) (Table 2). Our work supports the notion that activating regions can function independently of the nature of the DNA-binding domain, and reinforces the idea

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TABLE 1 Activating sequences derived from *E. coli* isolated as fusion proteins to LexA(1-202)

Name*	Length†	Charge‡	Activity§	Amino-acid sequence
B101	89	-9	480	ĒFPGIAĒFPT TĒAAĒASRĒK HGMNVLMGAP NĒVRGGHSGNVAASĒLAQL GLLĒILSDĒDY YPASLLĒDAAF RĒVADĒESNALRĒCAGĒEAGĒ
B102	41	-3	140	ĒFPGIGSISP FAĒDATMFVG TSDĒKIVPIMP WĒLLCTASVV L
B103	45	-9	120	ĒFPGIHVST FSKĒDRĒHĒID ĒEAĒĒĒEALĒE TĒLCLLWAMĒRĒISLQ
B104	98	-10	310	ĒFPGINDĒLR ĒARĒKLLLDĒIN ĒSGLPAAGĒEF LĒMĒIDĒĒTHA HLTPĒAĒILLS GĒRĒMGĒDVĒRS LLĒRĒNSALVĒD ĒCVAFYĒĒĒHF PĒDPSTCSQAN SĒGRĒISYĒDL
B107	80	-11	270	ĒFPGIPFSAS LTĒEQSYĒĒV DĒGĒSASMAĒEL CALISALAĒĒĒ PĒNQSIATG SVĒDPQNSAVC CHPĒDPSTCSQ ANSĒGRĒISYĒDL
B112	121	-16	1,200	ĒFPGITLRĒIQ ĒTĒDMLYĒKĒGĒDT LYĒLDWĒLEDGI ĒELVĒFDAPGS VNĒKLĒTAVA SLĒGĒAIGVLĒE QQSDĒLIWĒEL TVĒKĒAKĒVNFĒD SGLĒKĒĒĒĒEI PSADĒDFPVA ĒRĒSSGEĒFRĒ ĒRĒHSGGTĒDLC F
B114	219	-21	250	ĒFPGIQAQLH VĒRCADĒHQPDĒY NĒILSQRĒIQS SQĒRALGLVNLĒAĒĒTPVDVT SSMSMGWNFP LYĒEQVTGPV AALHYĒDGTĒTT SMYĒNĒFGĒDST TTLĒDRĒQPVĒĒ ĒĒĒNSTVYVP AVPSRĒKYWIS GĒAĒEGCLĒSĒG CSVVGĒĒĒĒEĒ MPGMYĒHGĒĒDY DVAGFCVGVV ĒĒKĒĒĒIDGSĒK VSDĒGDVĒLĒIAL GSSGPĒDHSNGY SLVRĒKĒĒĒĒVS GĒDPSTCSQA NSĒGRĒISYĒDL
LexA	0	0	<1	

To make full-length LexA fusion proteins, we made the vector LexA(1-202)+PL, which is identical to pMA424 except that *GAL4*(1-147) sequences are replaced with *LexA*(1-202) sequences¹. The *LexA* gene was flanked by the *ADH1* promoter and terminator. The DNA sequence of LexA(1-202)+PL from the 202nd codon of LexA is: 5'-CTG [GAAATTC]^E CCGG [GGATCC]^B [GTCGAC]^S [CTGCAG]^P-3', where E, *EcoRI*; B, *BamHI*; S, *Sall* and P, *PstI* restriction endonuclease recognition sequences, respectively. The underlined GAA, originally a UAA stop codon, and the following polylinker sequences were added by site-directed mutagenesis and standard cloning procedures⁶. As with the parent vector pMA424, the *EcoRI*, *BamHI* and *Sall* sites were unique in the vector LexA(1-202)+PL'. To generate plasmids capable of expressing LexA fusion proteins, *E. coli* genomic DNA was digested with *SauIII*A restriction enzyme and inserted into the *BamHI* site of the vector LexA(1-202)+PL. These plasmids were transformed into *E. coli*, and ~10,000 of the *E. coli* colonies were pooled and the plasmid DNA was isolated from the pool. More than 90% of the plasmids had an insert of *E. coli* genomic DNA. The plasmid DNA from the pool was transformed into the yeast strain YT6:lexΔ1(OPx2, +36) (see Table 2) and the resulting transformants were replica-plated onto plates containing minimal media lacking histidine and containing 2% (v/v) glycerol, 2% (v/v) lactic acid (pH 7.0), and 200 μg ml⁻¹ X-gal (5-chloro-4-bromo-3-indol-β-D-galactoside)¹. Roughly 1% of the yeast colonies turned blue overnight on the X-gal plates. The plasmids from seven blue colonies were isolated and the *E. coli* DNA inserts were sequenced several times in both directions after subcloning into plasmids containing the M13 origin.

* Activating region encoded by *E. coli* genomic DNA.

† Number of amino acids attached after the 202nd amino acid of LexA (the first five amino acids are encoded by the polylinker sequences, and the remainder are encoded by the *E. coli* genomic DNA).

‡ Net charge of the activating region.

§ Units of β-galactosidase enzyme activity produced when the activator is transformed into yeast strain YT6:lexΔ1(OPx2, +36) (see Table 2).

|| Amino-acid sequence is deduced from the DNA sequence and shown using the single-letter amino-acid code. +, Residues bearing a positive charge (R and K); -, residues bearing a negative charge (D and E). The origin of the B104 sequence residues 5-34, as determined by searching for the sequences in GenBank, is identical to *E. coli aroG* residues 119-148, the phenylalanine synthetase gene.

TABLE 2 *E. coli* activation sequences attached to DNA-binding regions

Name	Length	Charge	Activity		
			GAL4 (1-147)	LexA (1-87)	LexA (1-202)
B6	76	-8	122*	ND†	943‡
B7	42	-8	87*	11‡	1,461‡
B16	52	-6	80*	5‡	393‡
B17	28	-5	168*	1‡	167‡
B42	79	-10	271*	750‡	1,816‡
B112	121	-16	294*	ND†	2,140§
GAL4			2,080*§	1,649‡	ND†
None			<1	<1	<1

E. coli-derived activation sequences attached to GAL4(1-147), LexA(1-87), and LexA(1-202). B6, B7, B16, B17 and B42 were isolated from our previous screen for activating regions attached to GAL4(1-147), and B112 was isolated as a LexA(1-202) fusion (Table 1). The activating regions isolated on GAL4(1-147) were attached to LexA(1-87) and LexA(1-202); B112 was attached to GAL4(1-147).

* β-Galactosidase (units per mg total protein) assayed in yeast cells (YT6:pRY171 (MATa *gal4*-542 *gal80*-538 *ura3*-52 *his3*-200 *ade2*-101 *ade1* *lys2*-801 *trp1*-901 *aro1* *leu2*-3-112, *mel1*, Met⁻)⁶), which contained a *GAL1-lacZ* fusion gene integrated at the *URA3* locus and were transformed with plasmids capable of expressing various GAL4 hybrid proteins. The yeast cells were grown in minimal media lacking histidine and containing 2% (v/v) glycerol and 2% (v/v) ethanol to an A₆₀₀ of between 1 and 2. The β-galactosidase activities were assayed according to ref. 8 rather than by the method described in Table 1.

† ND, not determined.

‡ β-Galactosidase (units per mg total protein) assayed in yeast cells (YT6:lexΔ1(OP × 5)) which contained an integrated *GAL1-lacZ* fusion gene with five LexA operators in place of UAS₆ and were transformed with plasmids capable of expressing the indicated LexA hybrid proteins.

§ This protein is wild-type GAL4.

|| This protein is LexA(1-87)+GAL4(74-881) (ref. 7).

that the activating regions detected in our screens are exclusively of the acidic type. □

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Human γ -globin genes silenced independently of other genes in the β -globin locus

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ERYTHROPOIESIS during human development is characterized by switches in expression of β -like globin genes during the transition from the embryonic through fetal to adult stages. Activation and high-level expression of the genes is directed by the locus control region (LCR), located 5' to the ϵ gene^{1–3}. The location of the LCR and its role in directing high-level expression of the globin genes has led to the suggestion that competition from the β gene for interaction with the LCR has a major role in silencing the fetal γ genes during adult life^{4,5}. We have now constructed lines of transgenic mice containing the human $A\gamma$ globin gene linked to the LCR. We observe high-level expression of the transgene in the embryonic stages but silencing of the gene in adult animals. We conclude that the γ gene is not deregulated by the presence of the LCR and that competition from the β gene is not required for silencing of the γ genes in adult life. The silencing is therefore likely to be mediated by stage-specific factors binding to sequences immediately flanking the genes.

Two constructs (Fig. 1) were injected into mouse oocytes. Mini-LCR γ contained the $A\gamma$ gene and its enhancer region cloned between the complete 5' LCR sequence and the 3' hypersensitive site-1 region¹, whereas $\Delta 3'\gamma$ lacked the 3' site-1 region. Fifty-five founder animals were generated. To avoid artefacts from high copy numbers, end-blotting was used to exclude animals carrying more than two copies of the transgene. Six such lines were analysed as well as one line carrying three copies. Southern blots showed all integrated fragments were intact and arranged in a tandem repeat where more than one copy was present. Yolk sac RNA from 10.5-day embryos and blood RNA from adult animals was analysed for the presence of γ transcripts. Expression in embryonic yolk sac was compared with that of the mouse embryonic $\beta H1$ gene, whereas expression in adults was compared with the mouse β_{maj} gene (Figs 1 and 2a, and Table 1). In embryonic yolk sac the γ gene expressed on a per-copy basis at between one and two times the level of the $\beta H1$ gene. The relative level of embryonic γ transcription cannot be precisely quantitated as it was for the human adult β gene¹ because erythropoiesis at 10.5 days is in a dynamic state compared with the steady state of adult erythropoiesis. Two mouse β -like genes $\beta H1$ and $\epsilon\gamma$ are expressed in the yolk sac at 10.5 days. Between 10 and 12 days $\beta H1$ expression drops sharply, whereas $\epsilon\gamma$ expression increases before declining and disappearing from the circulation by 16.5 days⁶. The human γ -globin gene is normally expressed as a fetal gene so it is not possible to tell

which of the mouse genes it should most closely follow in patterns of expression. It does not seem to follow either $\beta H1$ or $\epsilon\gamma$ completely as expression is still detectable in 16.5-day fetal liver at levels of up to 20% of β_{maj} per copy (Fig. 2b).

Analysis of blood from adult animals bred from each of the γ transgenic lines showed that three lines expressed the transgene at less than 0.5% of the expression observed for the mouse β_{maj} gene (Fig. 2a and Table 1). Three other lines expressed at around 1% of total β_{maj} or 2% when corrected for the copy number of the β_{maj} gene. The line that contained three copies of the construct ($\Delta 3'\gamma$ line 4) expressed the transgene at a level of 6% per copy. Although the six lines carrying one or two copies express the γ gene at very low levels, it is clear that there is considerable variation in this basal level (see Table 1). These differences are reproducible for each line (two animals per line) and are not related to copy number. For example, the single copy mini-LCR line 2 shows higher levels of expression than the two-copy mini-LCR line 1. We conclude that these differences are due to integration position effects. Analysis of these two lines during the fetal stages (Fig. 2b) shows that γ transcripts are present in fetal liver, but at a lower level than in embryonic yolk sac (15–50% of mouse β_{maj}), and expression declines during development to the very low adult level. The difference in γ expression between the two lines in adults is also present in the fetal stages. Our results show that a γ -globin gene linked to the LCR is silenced in transgenic mice without a linked β -globin gene. But it is also clear that the γ -gene is susceptible to positive position effects which affect the basal level and, in the three-copy

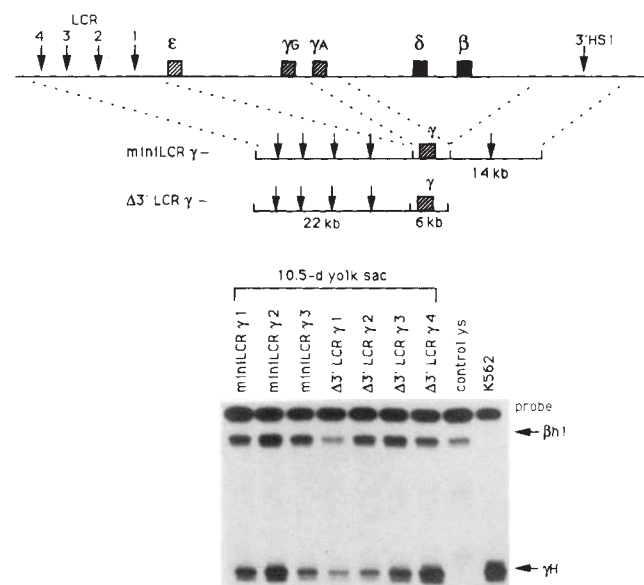


FIG. 1 S1-nuclease analysis of γ -globin transcripts in 10.5-d yolk sac from transgenic mouse lines containing the constructs shown. The probe mix contained two 5' end-labelled fragments, a *Bst*NI fragment (190 bp) mapping to the $A\gamma$ cap site, and a mouse $\beta H1$ *Hinf*I fragment mapping to the 5' end of exon 3. Probes were of equivalent specific activity.

METHODS. The mini-LCR γ construct was made by cloning a 5.6-kb fragment extending from $\sim 1,340$ relative to the $A\gamma$ cap site to $+4,308$ into a polylinker between *Clal* and *KpnI* sites and then exchanging the fragment for the β globin *Clal*–*KpnI* fragment of the minilocus¹. A *Sall* fragment containing the entire insert and a *Sall*–*KpnI* fragment lacking the 3' hypersensitive site 1 region were injected into mouse oocytes. Copy number of the resulting transgenic mouse lines was determined by Southern blot analysis of end fragments. The copy numbers of the lines are as follows: mini-LCR γ lines 2 and 3 and $\Delta 3'\gamma$ lines 1, 2 and 3, 1 copy; mini-LCR line 1, 2 copies; $\Delta 3'$ line 4, 3 copies. The new nomenclature and numbering used is that agreed at the Seventh Conference on Hemoglobin Switching, Airlie House, 1990.

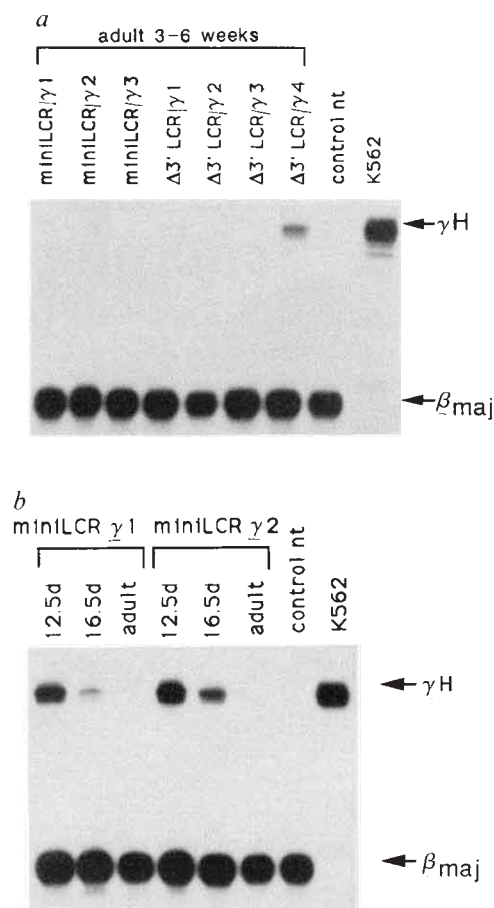


FIG. 2 S1-nuclease analysis of adult and fetal expression of γ -globin RNA in the transgenic lines described in Fig. 1. The same γ probe was used together with a 5' end-labelled *NcoI*-*HindIII* fragment from the mouse β_{maj} gene (700 bp) which maps the 5' boundary of exon 2. *a*, Analysis of blood RNA from adults aged 3–6 weeks bred from the same lines as in Fig. 1. *b*, Analysis of RNA from fetal liver (12.5 and 16.5 d gestation) and adult blood from two mini-LCR γ lines.

line, give rise to a significant level of adult expression. It should be noted that these position effects affect the silencing of the gene which is likely to be a promoter-mediated function. It has been suggested that the enhancer-like sequences located at the breakpoints in several of the deletional hereditary persistence of fetal haemoglobin (HPFH) syndromes interfere with γ silencing causing the high-level expression observed in these conditions. The position effects observed by us may be analogous to these^{8,9}.

TABLE 1 Relative levels of γ transcripts compared with β_{maj} levels at 10.5 d and β_{maj} levels in adults

	10.5 days γ/β_{maj} /copy (%)	Adult γ/β_{maj} /copy (%)
mini LCR $\gamma 1$	105	<0.5
mini LCR $\gamma 2$	200	2
mini LCR $\gamma 3$	128	<0.5
$\Delta 3'$ LCR $\gamma 1$	200	2
$\Delta 3'$ LCR $\gamma 2$	104	2
$\Delta 3'$ LCR $\gamma 3$	200	<0.5
$\Delta 3'$ LCR $\gamma 4$	170	6

See also Figs 1 and 2a. Quotation was by densitometric scanning and RNA dilution.

Our findings contradict earlier reports^{4,5} that the γ genes are deregulated in adult transgenic mice by the LCR and that competition from the β gene is required for silencing of γ transcription in adult stages. Several features of these studies might explain the observed adult expression. Enver *et al.*⁴ used a γ gene linked to a 2.6-kilobase (kb) micro-LCR construct, which is missing all of site 4 (S. Pruzina *et al.*, manuscript submitted) and part of site 3 (ref. 19). The construct does contain the complete site 2, which is the only site known to contain a strong enhancer activity^{10–12}. The small size of the construct resulted in the site-2 enhancer being ~2 kb upstream from the γ cap site compared with 11 kb in our construct and 25 kb in the normal human locus. Behringer *et al.*⁵, analysed adult expression in two lines co-injected with human α , β and γ genes. We have found that the structures adopted by co-injected genes are too complex to be resolved by conventional Southern blotting (P. Fraser, D. Whyatt and N.D., unpublished results). Co-injection of fetal and adult genes as used by Behringer *et al.* may bring the 3' end of the γ gene close to activating sequences associated with the adult α and β genes (for example, the β -globin 3' enhancer). In addition, in both of those studies, increases in adult γ expression may have been amplified in multicopy animals. The three-copy line in this study ($\Delta 3'$ line 4) demonstrates how a mild position effect can be exaggerated by even a relatively low copy number to give an apparently significant level of adult expression. Finally, the constructs used in those studies lack the region located at the 3' end of the γ gene which shows enhancer activity in transient expression assays¹³. It is present in our constructs suggesting that it might have a role in silencing the γ genes.

We have found that there is no deregulation of a γ gene in transgenic mice as a result of linkage to the LCR and that the presence of a β -globin gene in *cis* is not required for silencing of γ expression. The phenotypes of some human conditions involving deletions of the β gene have been proposed as support for a competition model¹⁴. Small deletions of the β promoter

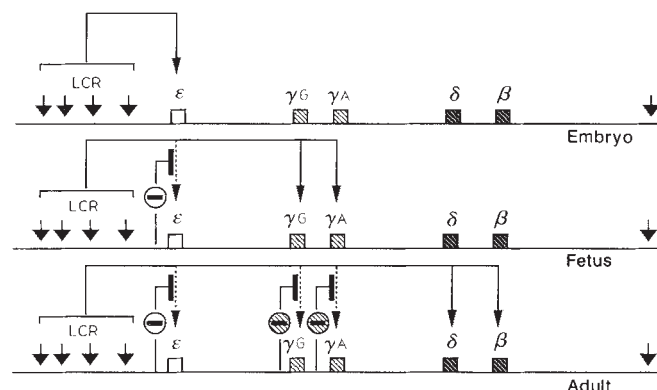


FIG. 3 Model for stage-specific regulation of the genes of the β -globin locus. Solid lines indicate activation of genes by the LCR. $\ominus$, Stage-specific negative factors silencing the gene. The location of these is not accurate and there may be more than one factor for each gene.

that abolish β transcription produce levels of γ expression averaging less than 5% (reviewed in ref. 15). Higher levels (averaged 10%) are only seen with much larger deletions (>10 kb) associated with the $\delta\beta$ thalassaemias¹⁵ and the distribution is heterogeneous with γ chains not detectable in many cells using existing staining methods¹⁶. This heterocellular distribution is problematic for a competition model based on the proposition that deletion of the β gene allows the LCR to exert a dominant positive effect on the γ genes in an adult environment. Changes in chromatin structure and/or position effects caused by these large deletions may directly interfere with promoter-mediated silencing. This seems more likely than a competition model. The high-level pancellular expression observed in the deletion HPFH conditions suggests that extra mechanisms operate in these syndromes. One possible mechanism is that the expression is the result of enhancer-like sequences which have been found close to several of the HPFH deletion breakpoints^{7,8}.

From our data, and other results for the ϵ gene (refs 17 and 18; and P. Watt and P. Fraser, unpublished results), it seems likely that both of these genes are silenced by sequences located in their promoters. But the relative ease with which silencing of the γ genes can be perturbed suggests that the precise mechanisms of operation of these sequences may differ. When the β gene is placed in a position relative to the LCR which is similar to that normally occupied by ϵ , it is expressed in the embryonic stage in transgenic mice (ref. 5, and O. Hanscombe *et al.*, manuscript submitted). Therefore early silencing of the β gene may depend on its precise position in the locus and on the presence of active genes between it and the LCR (O. Hanscombe *et al.*, manuscript submitted). A model based on these findings is illustrated in Fig. 3. □

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CORRECTION

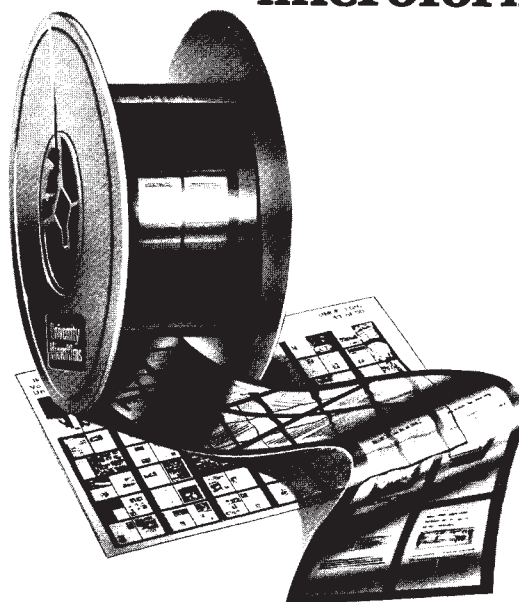
Spatial variability in the sink for atmospheric carbon dioxide in the North Atlantic

Andrew J. Watson, C. Robinson, J. E. Robertson,
P. J. le B. Williams & M. J. R. Fasham

Nature **350**, 50-53 (1991).

THIS letter appeared in the 7 March issue with the third author's name shown incorrectly. The correct form is as above.

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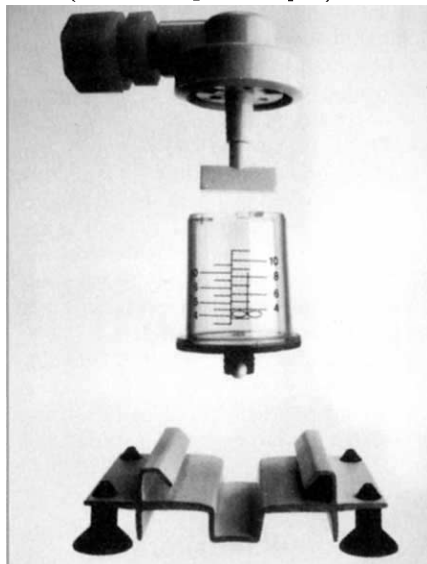
patible with most biological buffers and commonly used buffer additives, including chaotropes, detergents, chelating agents and thiols. They are stable in the pH range of 3–13 and can be used intermittently with solutions in the pH range of 2–14.3.

Fine filters

SpinTrex, a new concept in rotary membrane separation technology from New Brunswick Scientific, can now be integrated with the company's BioFlo Series of benchtop fermenters to provide a complete bioprocessing system (*Reader Service No. 101*). This complete package provides the user with a system that can be used to grow high-density cultures and then to recover high concentrations of viable cells, proteins and other by-products. It works as follows: the microbial culture is fed into the SpinTrex cylindrical separation chamber, which contains the rotating membrane cartridge. Rotation of the filter between 200 and 4,000 r.p.m. produces Taylor Vortices, which impart a gentle scrubbing action on the membrane. By continuously carrying particles away from its surface, "fouling" of the membrane is minimised, says NBS. Biomass is separated from broth, or macromolecules are separated from smaller molecules in the annular

gap between the separation chamber and the membrane. The SpinTrex set-up can achieve process rates of up to 80 litres per day and with a hold-up volume of only 10 ml. Smaller batches of 35 ml or less can be processed. The system is microprocessor-controlled and designed for unattended operation. NBS can supply a range of ultrafilters and microfilters to suit most bioprocessing applications.

Filtron offers Stirred Cell Systems for small-volume laboratory tangential filtration (*Reader Service No. 102*). The stirred cells are comprised of both reusable and disposable components: a pressure cap with magnetic stirrer, suction-cup base, storage base/holder and tube fittings are reusable; the stirred cell, storage and outlet caps, and tubing are disposable. Stirred cell starter kits are available containing cells with 10-ml and 150-ml capacities. The cells are available with three types of polyethersulphone membrane (Nova, Omega and Alpha) and with a



Stirred cells come in 10- and 150-ml capacities from Filtron.

wide selection of ultrafiltration and microfiltration pore sizes. The need for handling membranes or manipulating Orings is eliminated as the membrane is ultrasonically sealed to the membrane support base. The magnetic stir bar provides a constant tangential flow motion over the surface of the membrane. For ease of identification, the stirred cells are colour coded.

In a spin

Beckman has added a benchtop version to its Optima Series of ultracentrifuges (*Reader Service No. 103*). The new Optima TLX is



Beckman's TLX ultracentrifuge packs up to 120,000 r.p.m. into a benchtop unit.

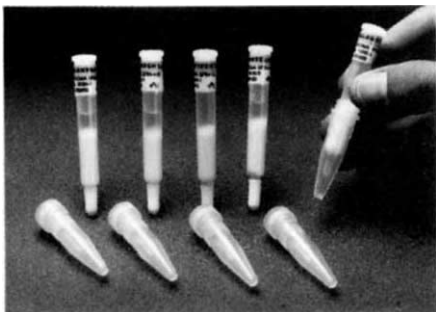
compact enough to fit underneath a fume hood and yet offers a spin speed of up to 120,000 r.p.m./625,000 g, says Beckman. The TLX can be supplied with a range of rotor types: fixed angle, vertical tube, swinging bucket and the latest NVT (Near Vertical Tube) rotor. The ultracentrifuge is designed to allow the separation of proteins in 55 minutes, subcellular fractionation in 20 minutes, separation of viruses in 60 minutes, membrane separation in 15 minutes and DNA and RNA separations in 2.5 hours and 1 hour, respectively. Safety features include fixed-angle rotors fitted with a fluid-containment annulus for containing spills from sample tubes and an alarm system that alerts users in the event of power failure, rotor overspeed, loss of vacuum, overtemperature, an overheated drive, rotor imbalance or an unlocked door. Standard features of the TLX include an imbalance-tolerant drive, ten different start/stop profiles that protect shallow gradients and a thermoelectric cooling system that eliminates the need for chlorofluorocarbons.

High speed and high capacity are features of the SR12.22 high-capacity centrifuge from Jouan, which is designed for batch centrifugation procedures (*Reader Service No. 104*). The SR12.22 is designed for the centrifugation of greater sample volumes at higher RCF: $6 \times 1,000$ ml at 14,000 g or 6×500 ml at 25,600 g. When defining run protocols, the user can programme the speed or RCF, time or integral, centrifugation radius, temperature, rotor chamber vacuum and brake rates. Up to 100 run programs can be stored. Safety features include a dual safety-lid lock, imbalance detection and shutdown, motor and rotor chamber overtemperature detection and shutdown, overspeed detection and vacuum level detection. Temperature control from $\sim 8^\circ\text{C}$ to $+40^\circ\text{C}$ and easy to clean stainless steel chamber walls are provided.

Centrifugal concentrators

The Centriprep-100 concentrator from Amicon is a handy membrane device for separating proteins from cells (*Reader Service No. 105*). It works as follows: when spun in a centrifuge, centrifugal force drives suspended materials away from the membrane while membrane-permeating species and solvent are propelled through the membrane, unimpeded by accumulated material that is retained on its surface. Amicon says, Centriprep-100 is particularly useful for the separation of low molecular weight proteins from cells, cell debris or large proteins in solution. Additional uses include the processing of cell broths and the concentration of virus and large proteins. Centriprep-100 is one of a series of devices that have a 15-ml capacity. Membranes with molecular weight cut-offs of 10,000, 30,000 and 100,000 are available.

Clontech has a new line of Chroma Spin columns, which are designed for the **purification and size-fractionation of nucleic acids** in less than five minutes (*Reader Service No. 106*). Five column sizes are available for the removal of DNA fragments smaller than 10, 30, 100, 400 or 1,000 bp.



Clontech's nucleic acid spin columns.

The need to transfer samples after elution is eliminated because the Chroma Spin columns fit into microcentrifuge tubes. Intended applications include the removal of unincorporated nucleotides from labelling reactions and the removal of unextended PCR primers. The columns can provide greater than 95 per cent removal of unincorporated nucleotides, says Clontech. For large DNA fragments, recovery rates of greater than 90 per cent can be achieved. Each column is supplied with two RNase-free microcentrifuge tubes. The columns are sold in packs of 20 columns.

Polysciences has introduced two new devices to its CentriCell line of **ultrafiltration devices**, which are intended for concentrating, fractionating or desalting biological samples (*Reader Service No. 107*). The Centrifuge Concentrator, which is designed to fit into centrifuge carrier tubes (17 × 120 mm), can hold starting volumes of 0.4 ml to 2.5 ml. On the other hand, the Centrifuge Micro-Concentrator holds a starting volume of 20 µl to 400 µl and fits in Eppendorf-style rotors or buckets. The hydrophilic cellulose ester membranes exhibit low protein adsorption and provide high sample recovery, says

Polysciences. Both sizes of concentrator are available in packs of 24 or 100. Both devices are available with two molecular weight cut-offs: 10,000 or 50,000 Da.

Plasmid purification

Nucleobond AX Cartridges from The Nest Group are designed for the quick and easy **purification of DNA or RNA plasmids** (*Reader Service No. 108*). The new AX cartridges offer high capacity for larger scale isolations, for example, 1,200 µg of DNA, 2,000 µg of plasmid DNA or 8,000 µg RNA. According to the company, recovery rates of 85–100 per cent can be achieved, depending on the application. The cartridges contain a macroporous silica matrix that is bonded with a hydrophilic anion-exchange coating; this serves to minimise nonspecific adsorption and promote high mass recovery. Nucleobond AX cartridges are compatible with syringes with Luer connectors or a vacuum manifold. They are available in a variety of sizes and are reusable where appropriate.

5 Prime → 3 Prime has developed a range of **spin columns for plasmid DNA purification** of cultures from 1 ml up to 1 litre (*Reader Service No. 109*). Go from RNase-digested cleared lysates to purified plasmids in only 20 minutes, the company claims, without using CsCl gradients or performing phenol extractions. The user prepares cleared lysates using the recommended methods, loads the column, spins through the washes and spin elutes the plasmid. Plasmid purified in this way can be used for restriction digestion, sequencing, transfection, transformation, ligation and *in vitro* transcription. Plasmid Select kits contain RNase Plus, an optimized blend of bovine pancreatic RNase A and RNase T1, the buffers and reagents that are required for the preparation of cleared lysates, column washes and elution, and the necessary processing and collection tubes. A Plasmid Select-10 kit for 1–10-ml cultures costs \$110 (US) and contains 20 columns. A 10-column Plasmid Select-100 kit for 10–100-ml cultures costs \$100 (US). For larger volumes in the 200–1,000-ml range, the three-column pZ523 kit costs \$55 (US).

Gels and gel markers

A combination of high gel strength and low electroendosmosis values are features of the pulsed-field gel electrophoresis (PFGE) grade agarose developed by Boehringer for the separation of high molecular weight DNA (*Reader Service No. 110*). PFGE-grade agarose provides a gel strength of greater than 3,000 g cm⁻² and an electroendosmotic value of less than 0.012; this facilitates the preparation of gels of less than 0.08 per cent and reduces the long run times that are typically associated with pulsed-field applications, says the company.

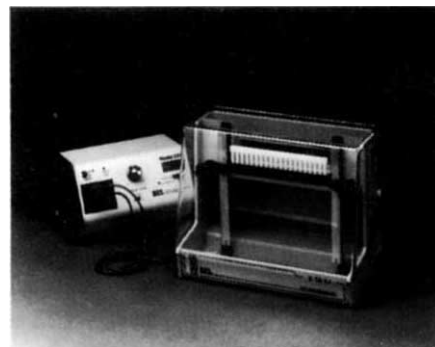
Chromosomal Grade Agarose, which Bio-Rad claims has the highest gel strength

of available electrophoresis agarose products, is designed specifically for **megabase DNA separations** (*Reader Service No. 111*). The high gel strength (6,000 g cm⁻²) permits the preparation of very low percentage gels. It is the high gel strength and sieving properties of Chromosomal Grade Agarose that allow megabase DNA separations to be performed up to 40 per cent faster than with conventional agarose. For example, the separation time for *Schizosaccharomyces pombe* is reduced by four days, says Bio-Rad. The company notes, however, that as DNA size decreases to below 1 Mbp, the difference in separation times between Chromosomal Grade Agarose and conventional agarose diminishes.

For user convenience, International Biotechnologies has introduced an **RNA marker** that is supplied in a ready-to-load buffer and which is compatible with most RNA gel systems (*Reader Service No. 112*). The RNA marker is made up of eight transcripts ranging from 9.5 K to 0.363 K.

Electrophoresis hardware

For **protein and nucleic acid separations** in a 15 × 17-cm vertical gel format, Gibco/BRL has introduced the V15 17 Vertical Gel Apparatus (*Reader Service No. 113*). The



A quick-assembly vertical gel apparatus from Gibco BRL.

apparatus consists of the basic electrophoresis unit with a safety lid to protect against accidental electrical contact during a run, integral gel clamps and power cords. In addition, there are two glass plates — one short (16 cm × 19.7 cm), one long (18.5 cm × 19.7 cm) — with a pair of side spacers, a bottom spacer and a 20-tooth, well-forming comb.

In designing BaseAce, Stratagene's new **sequencing apparatus**, the company has tackled some of the common problems associated with vertical gel electrophoresis — buffer leakage, the cracking of plates through stress and difficulties with buffer disposal (*Reader Service No. 114*). The gasket design creates a leak-proof seal and eliminates the need for notched gel plates or rubber spacers, says Stratagene. Sliding rubber roller clamps are used to hold the gel plates in place without exerting undue pressure upon them. In addition, as the gel plates utilize the entire format of a 14 × 17-inch



The BaseAce unit has a gasket design to create leakproof seals.

X-ray cassette, the number of lanes that can be loaded on a single gel is maximized. Other features include a thermal dispersion plate to ensure that all the lanes run straight and a buffer chamber that can easily be drained into a transportable lower chamber. The basic BaseAce sequencing unit costs \$795 (US).

By incorporating an anodised aluminium back plate into the design of Hybaid's new sequencing gel tank, the company hopes that this will provide a more even heat distribution across the gel and eliminate the problems of uneven migration and band smearing (*Reader Service No. 115*). Sequence gels (45 cm × 35 cm) are held in place by two retaining bars. This ensures an even pressure distribution along the length of the gel and minimises both leakage and plate breakage, says Hybaid. The upper buffer chamber of the gel tank drains into a separate drain sink at the back of the apparatus, and both the lower buffer chamber and drain sink are removable for the purposes of decontamination and cleaning. With safety in mind, the unit also incorporates interlocking safety lids that ensure the electrodes are connected only when the buffer chambers are closed. The sequencing system includes gel plates, spacers and a 78-tooth sharktooth comb.

Assorted columns

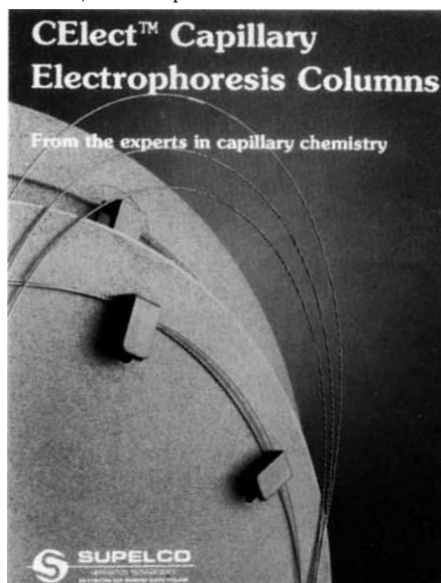
Mitsubishi Kasei has introduced a new **chiral chromatography column** for amino acid and hydroxy carboxylic acid D,L separation



Enantioselective columns from Mitsubishi.

(*Reader Service No. 116*). The MCI GEL CRS10w chiral column is based on an *N,N*-dioctyl-L-alanine coated 3 μ m, 100 Å bonded stationary phase. The chiral column can be used for the direct optical resolution of more than 20 different types of amino acids, including the separation of four isomers of allo-DL isoleucine and DL-isoleucine, at room temperature. Other stated uses include the analysis of trace impurities in D,L amino acid mixtures, hydroxy carboxylic acids and related compounds.

Capillary electrophoresis columns with three new novel phases are now available from Supelco (*Reader Service No. 117*). The new CElect columns offer a stable interior surface, which is preferable to bare silica tub-



Supelco's capillary electrophoresis columns feature three novel phases.

ing for many protein and polymer separations, says Supelco. The three phases include a moderately hydrophobic phase, a highly hydrophobic phase and a hydrophilic phase. Supelco recommends the use of the hydrophobic columns for increased selectivity in micellar separations. All three columns are available in 50- μ m and 75- μ m internal diameter (ID). Supelco will also prepare custom products in 25- μ m and 100- μ m ID. Kits are available, which include one 50- μ m or 75- μ m ID column of each phase, an untreated silica column and a test standard.

Arrow Scientific is the UK distributor for the new range of **biocompatible glass chromatography columns** for analytical, preparative and pilot-scale applications that are manufactured by the German company Kronwald Separationstechnik (*Reader Service No. 118*). Glass columns with internal diameters ranging from 3 mm to 300 mm and lengths from 50 mm to 1,500 mm are available; all are made with Teflon end pieces and adaptors. Low-, medium- and high-pressure versions are available. Maximum pressure tolerances range from 6 bar to 100 bar, depending upon size. Arrow will also supply



Biocompatible glass columns covering analytical, preparative and pilot scale.

the glass columns prepacked with the media of your choice.

Chromatography currents

LDC Analytical has developed a new **multiple solvent delivery system**, called the constaMetric 4100, which is designed for low pressure gradient HPLC systems (*Reader Service No. 119*). The constaMetric 4100 has the capability of mixing four solvents and is suitable for isocratic mixing, binary, ternary and quaternary gradients, and column regeneration. The computer-designed cam ensures virtually pulse-free delivery of selected flow rates up to 6,000 p.s.i., regardless of downstream pressures, says LDC. The pump provides flow rates from 0.01 ml min⁻¹ to 10 ml min⁻¹ in 1- μ l increments and with a flow-rate precision of 0.1 per cent RSD. The £7,250 (UK) constaMetric 4100 is supplied with an RS232 communications port as standard for PC communication and control.

Adding to its existing HPLC product range, Severn Analytical has introduced a new **ternary gradient solvent delivery system**, the SA 6480, which can be used for microbore, analytical and preparative gradient HPLC (*Reader Service No. 120*). The £5,950 (UK) SA 6480 pump is designed for virtually pulse-free flow over the range of 1 μ l min⁻¹ to 50 ml min⁻¹. According to Severn, precise piston travel, a new ring seal material and rear seal washing are features that help to provide trouble-free delivery of even the most concentrated buffer. RS232 and IEEE interfaces allow the pump to be linked up to chromatography equipment from other manufacturers. Preparative pump heads for flow rates of up to 50 ml min⁻¹ and biocompatible titanium versions for the delivery of highly concentrated, corrosive eluents up to pH 14 are optional.

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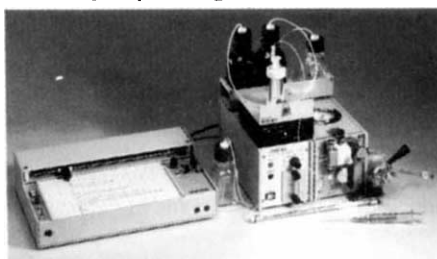
According to Gilson Medical Electronics, its new **programmable variable wavelength UV detector**, the 117 UV Detector, offers a maximum sensitivity of 0.001 AUFS in both single- and dual wavelength modes (*Reader Service No. 121*). The key to the high sensitivity is the digital signal processor for signal conditioning, says Gilson. The detector's sensitivity ranges from 0.001 to 200 AUFS, depending upon the type of flow cell used. Flow cells for analytical, microbore and preparative flow rates are available; all are



Maximum sensitivity in both single- and dual-wavelength modes — see Gilson's programmable UV detector.

interchangeable. The operating modes — single wavelength, dual wavelength and scan — and all operating parameters are programmed via the front keypad. All parameters — wavelength, sensitivity or operating mode — can be programmed to change during the run. The \$7,300 (US) 117 UV Detector can store up to nine method files. In addition, it has three mode-specific data outputs to interface with various recorders and data systems, and an in-built analog-to-digital converter, which can accept input from another detector for direct digital transmission to a Gilson data system.

The modular Mini-HPLC System from Knauer offers researchers a complete HPLC system for isocratic and gradient separations (*Reader Service No. 122*). Although the components can be purchased separately, the complete DM10,923 (Germany) Mini-HPLC System consists of a minipump, gradient former, injection valve (20-µl sample loop), injection syringe, Vertex column (Eurosphor C18), LC-photometer (254 nm fixed-wavelength detector) and single-channel chart recorder. The minipump can provide flow rates of 0.5 ml min⁻¹ to 5 ml min⁻¹, with a reproducibility of 0.2 per cent, says Knauer. Piston backflushing, a built-in bubble trap, check valves, a manometer and manual control of flow rates are features of the minipump. The gradient former is de-



Knauer's mini-HPLC system.

signed for the reproducible setting of the mixing volume of two solvents via a three-way valve.

New product listings

The 1991 **electrophoresis catalogue** features new product news from Integrated Separation Systems (*Reader Service No. 123*). Product listings include precast polyacrylamide gels, agarose gels, stains, products for SDS-PAGE, DNA sequencing, blotting and two-dimensional electrophoresis. Useful technical tips are provided, along with the product information and price list. A list of technical protocols is available on request.

Chromacol's 40-page 1991 **catalogue** of vials, caps, seals and crimpers for all areas of chromatography and capillary electrophoresis, is now available (*Reader Service*



Chromatographer's guide to new products from Chromacol.

No. 124). This handy reference guide contains compatibility charts to assist the user in matching the right consumables to the right instrumentation and application.

Product catalogue profiles are now available containing physical property descriptions and test data about the high performance silicone septa that are manufactured by Specialty Silicone Products (*Reader Service No. 125*). Twenty seven distinctive septa products are offered within four categories, including septa designed for use with gas chromatographs, autosamplers, water samplers and a variety of laboratory capliner applications. The septa range in thickness from 0.035 inches to 0.125 inches.

The free 240-page 1991 Hewlett Packard chromatography users catalogue is now out (*Reader Service No. 126*). It includes columns for gas and liquid chromatography, syringes, supplies for GC, HPLC, UV/VIS, FTIR, GC/MS and LC/MS instrumentation. ☐

These notes are compiled by Diane Gershon from information provided by the manufacturers. To obtain further details, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.